be formed between the central processes of neurones growing from opposite stumps of the severed connective. The fact that the conduction of action potentials is regained across the neuroma[15] and that some regenerating fibres grow beyond this structure (our unpublished work) suggests that axons also restore their specific connections at the ganglionic neuropile. Hence, a given pair of neurones of successive ganglia might renew their interconnections completely either at one or both neuropiles. Alternatively, many of the synapses found at the neuroma may be duplicates of those formed by the same regenerating fibres at the level of the ganglia. Thus, there would be a factor of redundancy in regenerated synapses. This might explain some consistent differences between regenerated and normal synapses with regard to the balance of excitation and inhibition[17].

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Effect of prostaglandins E_1, E_2 and $F_{2\alpha}$ on human blood coagulation

As prostaglandins are being used to induce labour and abortion in some women, it is necessary to know the effect of these compounds on the blood coagulation system. We have investigated this problem using plasma from normal women and prostaglandins E_1, E_2 and $F_{2\alpha}$ (Upjohn).

The prostaglandins, dissolved in absolute ethanol, were added to samples of the plasma to give a final concentration of 1, 10 and 100 ng ml^{-1}. Each 0.1 ml of plasma contained the equivalent of 1.0 ng of ethanol, which was added to similar samples of plasma during control runs of coagulation tests. After the addition of prostaglandins, aliquots of plasma were incubated for 1 h at 37° C to determine whether there was any conversion to prostaglandins A_1 or B_1[1,2]. While preparing 'platelet-rich plasma' we minimised the release of prostaglandin from the platelets. We used slightly modified versions of established methods to study blood coagulation in ten samples of plasma from each of ten subjects. To 0.2 ml of control and prostaglandin-treated plasma we added one of the following: thromboplastin, to measure prothrombin time[3]; cephaloplastin (Dade, Miami) to measure partial thromboplastin time[4], and thrombin (0.7 U per 0.1 ml, Fibrinex, Ortho) to measure thrombin clotting time. Percentage clot retraction was estimated by addition of 0.1 ml of cephaloplastin to 0.8 ml of platelet-rich plasma, followed by 0.1 ml of 0.1 M $CaCl_2$ in 1.0-ml graduated tubes into which were inserted applicator sticks. This mixture was incubated for 30 min at 37° C. After removal of the clot adhering to the stick, the remaining fluid was estimated as the percentage of the original 1.0 ml.

Plasma factors, V, VII, VIII, IX, X, XI and XII were determined by a modification of the test for prothrombin and partial thromboplastin time, using plasma from subjects deficient in the various factors as the vehicle for the clotting effect on control and prostaglandin-treated plasma. The plasma factor assays for the several prostaglandins *in vitro* were assessed at the 100 ng level only. Coagulation tests were also carried out on plasma from several pregnant women receiving prostaglandin $F_{2\alpha}$ or Pitocin for induction of labour, or Pitocin for abortion, and from women at the time of delivery (unpublished results of R. K. Laros).

None of the prostaglandins in concentrations of 1–100 ng ml^{-1} affected the coagulation tests or the plasma factor assays. Similarly, none of the plasma from the *in vivo* study (unpublished results of R. K. Laros) yielded any significant deviation from normal values for prothrombin time, partial thromboplastin time, thrombin clotting time or percentage clot retraction. *In vivo* studies on patients receiving prostaglandin $F_{2\alpha}$ as a means of inducing abortion also independently supports this conclusion[5]. None of the plasma samples converted the added prostaglandins to A_1 or B_1, although this has been described elsewhere[1]. Tables 1 and 2 summarise the *in vitro* findings.

Table 1 Effect of prostaglandins E_1, E_2 and $F_{2\alpha}$ on coagulation tests on human blood plasma

Prostaglandin	ng	Mean* PT (S)	Mean† PTT (S)	Mean‡ TCT (S)	Mean§ clot retraction
E_1	1	10.2	31.3	8.8	83.0
E_1	10	10.1	30.3	8.8	80.0
E_1	100	10.2	32.5	8.8	70.0
E_2	1	10.1	32.0	8.3	80.0
E_2	10	10.3	32.2	8.8	80.0
E_2	100	10.3	32.5	8.8	85.0
$F_{2\alpha}$	1	10.0	31.0	8.8	85.0
$F_{2\alpha}$	10	10.2	32.0	8.8	75.0
$F_{2\alpha}$	100	10.3	31.0	8.3	80.0
Mean Control		10.2	30.5	8.8	80.0

Clot retraction is expressed as percentage of control. PT, Thromboplastin time; PTT, partial thromboplastin time; TCT, thrombin clotting time.
* Range, 10–16 s.
† Range, 30–33.0 s.
‡ Range, 8.0–9.0 s.
§ Range, 70.0–90.0%.

Prostaglandins E_1 and E_2, apparently at physiological (1.0 ng ml^{-1} plasma) as well as the pharmacological levels we used, do not affect the clotting mechanism other than by inhibiting platelet aggregation and adhesiveness on the part of prostaglandin E_1. Blood samples from patients obtained before infusion of prostaglandin $F_{2\alpha}$ and at delivery, when prostaglandin concentrations are maximum and immediately *post partum* were relatively unchanged, although significant coagulation abnormalities developed in patients being aborted with the aid of Pitocin (unpublished results of R. K. Laros).

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Table 2 Effect of prostaglandins E_1, E_2 and $F_{2\alpha}$ on plasma coagulation factors tests

Prostaglandin	ng	Mean factor V	Mean factor VII	Mean factor VIII	Mean factor IX	Mean factor X	Mean factor XI	Mean factor XII
E_1	100	104	100	73.0	90.0	100	102.0	88.0
E_2	100	101	147	117.0	87.0	113	83.0	92.0
$F_{2\alpha}$	100	104	100	77.0	90.0	100	96.0	106.0
Control (1 ng ethanol)		100	100	100.0	100.0	100	100.0	100.0

Values are expressed as mean percentage of control.
Ranges for factors: V, 100–108; VII, 100–150; VIII, 70-110; IX, 90–100; X, 100–106; XI, 100–104; XII, 80–100.

Prostaglandins seem to affect haemostasis through the platelet. The ability of prostaglandins to interfere with platelet aggregation has been well established and may represent one of the major effects of prostaglandins on the clotting mechanism, although less well documented. Ferri *et al.*[7] noted a shortening of the recalcified clotting time in citrated rat blood, suggesting the development of a hypercoagulable state. Kloeze[9], on the other hand, also working with rat plasma could not alter the prothrombin time, but changed the thromboelastogram pattern of prostaglandin-containing blood, an effect probably related to platelet function. In contrast to our findings, Murer[9], comparing different systems of clot retraction, noted an inhibitory effect of prostaglandin E_1 on diluted calcium-deficient plasma solutions clotted with thrombin. Addition of calcium reversed the inhibition, resulting in normal clot retraction. Suspensions of washed platelets where clots were formed by fibrin monomers and calcium also were inhibited by prostaglandin E_1 and were not improved by further addition of calcium[9]. Addition of calcium to the platelet-rich plasma alone in our system was unaffected by prostaglandins E_1, E_2 and $F_{2\alpha}$.

Haemostasis therefore does not seem to be altered significantly by prostaglandins E_1, E_2 and $F_{2\alpha}$, and thus haemorrhagic or thrombotic complications would not be expected to represent a hazard in their therapeutic use.

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Biochemical marker in dominantly inherited ectodermal malformation

ALTHOUGH several hundred dominantly inherited human malformations have been described[1], the mode of action of the mutant genes responsible for them is, in nearly all cases completely unknown, because of the lack of suitable biochemical markers. Here we describe a well defined biochemical abnormality in the keratin of mice bearing the dominantly inherited Naked trait.

The mouse phenotype known as Naked (N) has been described elsewhere[2–4]. In the heterozygote, the hair breaks off close to the root causing patchy depilation which begins around the eyes and spreads to the tail. Before depilation is complete, hair growth begins again on the head, and the mouse grows successive waves of hair passing from head to tail, reflecting the normal moult pattern. Mice homozygous for this mutation, have almost no hair or nails, and usually die within 10 d of birth.

We have investigated the amino acid composition of Nn hair and found it to be significantly different from that of wild type hair. We used mice of the NS-FR strain, inbred by brother–sister mating for 57 generations with forced heterozygosis for the N and albino genes. Eight mice were examined. Four were heterozygous Naked (Nn) and four were homozygous for the normal allele (nn). Each of the groups comprised two black and two albino mice.

Three replicate samples of hair shaft (2 mg each) were taken from each mouse, washed with water and defatted in petroleum ether, dried and added to 5 ml of redistilled 6 N HCl in a hydrolysis tube, which was then evacuated, sealed and heated at 110° C for 24 h. The contents of the tubes were then evaporated under vacuum to dryness and the residues were dissolved in *p*H 2.2 citrate buffer at a concentration of 0.15 mg of protein ml^{-1}. The amino acid composition of the resulting solution was determined on a Jeol analyser. A standard mixture of amino acid was analysed at every sixth run.

The amino acid composition of the hair samples (Table 1) is expressed as the percentage of moles represented by each amino acid. An analysis of variance was carried out to determine the effect of the N mutation, the albino mutation and the interaction between them. The albino gene causes no significant change nor does it interact significantly with the N mutation. The latter, however, causes two significant changes in amino acid composition a 30.4% reduction in the tyrosine content (3.23 against 4.67 mol %) and a 20.4% reduction in the glycine content (8.41 against 10.51 mol %). Since the probability of finding n or more differences, at significance P or less among N variables is $p^n.N!/(N-n)!n!$, the probability that this is a chance finding is 0.00015. A few samples of hair were hydrolysed for 48 and 72 h to estimate rates of amino acid destruction. The corrected values for glycine (mol %) are: normal 9.37, naked 7.72, and for tyrosine: normal 4.58, naked 3.25. The differences between genotypes thus persist when values are corrected for destruction. For these reasons, observed reductions in tyrosine and glycine are almost certainly the result of the mutation.

In 1964, Dedeurwaerder *et al.*[5] extracted from wool a protein fraction containing 27 mol % of glycine and 11 mol % of tyrosine. The proteins comprising this fraction are now known as the high-gly, high-tyr (HG) proteins. It was apparent from electron microscopic studies of the wool after extraction of the HG proteins, that they are located in the intercellular membrane complex. In fact, the removal of HG proteins releases intact cells from the fibre. A complete sequence of an HG protein has now been published[6]. It has a molecular weight of 6,950 and contains 23% glycine and 19% tyrosine.

It has been shown that the HG protein content of a hair is roughly proportional to its tyrosine content[7] and, extrapolating from keratin samples whose tyrosine content and HG protein content have both been determined, we estimate that murine hair contains 20–25% by weight of HG proteins. Murine hair contains more tyrosine than any animal hair so far investigated[8].

The composition of murine HG protein has been determined[8]. It contains roughly three times as much glycine and four times as much tyrosine as whole hair. A defi-

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