Kestrels were observed near 27 experimental nest-boxes. They hunted preferentially near nest-boxes where artificial trails had been created using vole urine and faeces (Fig. 3*a*). In contrast, hunting kestrels largely avoided areas near boxes with trails but no urine and faeces, or with no trails (Fig. 3*a*). Thus, kestrels were not using the trails themselves as cues for hunting. Paired kestrels also tended to occupy boxes near trails treated with vole urine and faeces, whereas resting kestrels chose boxes irrespective of treatment (Fig. 3*a*). Male kestrels provide for their mates from pair formation onwards, during which time females do not hunt but remain near their nest-box in preparation for egg-laying[10,16]. Therefore, the number of male kestrels hunting near boxes was greater than the number of females. This may explain why treatments differed in the number of hunting kestrels, significantly for males (which preferred to hunt near urine- and faeces-treated trails) but only marginally for females (Fig. 3*b*). In addition, the four rough-legged buzzards (*Buteo lagopus*) seen hunting near nest-boxes were all near sites treated with vole urine and faeces (Fig. 3*b*).

We have provided the first experimental evidence, to our knowledge, of a wild raptor using vole trail marks to select hunting patches and potential nest-sites. We suggest that scent marks of voles act as visible cues to kestrels, especially in spring when these are not covered by grass. In the laboratory experiment, we did not detect any peak of visible light in the ultraviolet arenas that might explain our results (Fig. 4). It is known that mouse urine fluoresces in blue[13], and we have found a dim blue fluorescence in the urine and faeces of voles. It is possible that the ability of kestrels to detect vole scent marks may not depend entirely on ultraviolet vision. The faint fluorescence is just visible to a human spectator when ultraviolet lamps are the only source of light: light from ordinary 60 W bulbs masks it completely. At Alajoki during our field experiment, the visible sunlight was so strong that any fluorescence was totally masked; we therefore consider it unlikely that fluorescence was the reason for our results. An alternative explanation, that kestrels detect vole scent marks by olfaction, is also unlikely, as in our laboratory experiment kestrels were able to distinguish vole scent marks in ultraviolet but not visible light. The biological significance of ultraviolet vision in higher vertebrates is poorly understood: it may play a role in orientation[17,18], food detection[19,20] and intraspecies communication[21,22] (reviewed in refs 23, 24). The absorbance or reflection of ultraviolet light by a food item could make it more conspicuous to a consumer. Although the eye structure of diurnal raptors (order Falconiformes) has not been tested systematically for sensitivity to ultraviolet light, most other diurnal birds studied so far have proved to be sensitive[24–26]. We therefore propose that, in the presence of ultraviolet light, diurnal raptors can easily see areas stained with fresh vole urine and faeces, and that they use these marks as visual cues when searching for areas of vole abundance. This ability would enable raptors to evaluate large areas in a relatively short time and would explain how nomadic raptors find patches of high vole abundance without prior knowledge of local food conditions. It has been suggested that scent marks of other vertebrates may also be visible in ultraviolet light[27], so this kind of hunting-site detection may not be uncommon. □

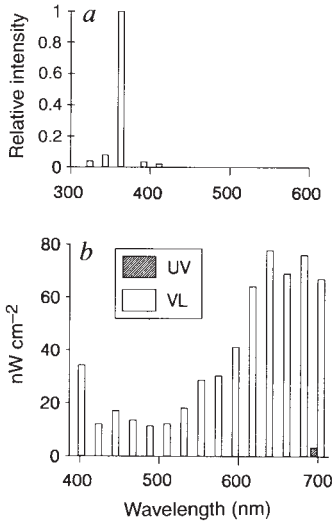

FIG. 4 *a*, Relative spectral energy distribution of the UV lamp (Philips MLW 160 W, 220–240 V) according to the manufacturer. *b*, The irradiance spectra between 400 and 700 nm on the bottom of UV arenas (UV) and visible light (VL) arenas measured with a series of interference filters (each penetrating ~15-nm band) immediately below the UV and VL lamps, respectively. The small amount of VL emitted by UV lamps (410 nm in *a*) was not measurable using our gauge.

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Corticotropin-releasing hormone deficiency reveals major fetal but not adult glucocorticoid need

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THE body responds to stress by activation of the hypothalamic–pituitary–adrenal (HPA) axis and release of glucocorticoids. Glucocorticoid production in the adult regulates carbohydrate and amino-acid metabolism, maintains blood pressure, and restrains the inflammatory response[1]. In the fetus, exogenous glucocorticoids accelerate maturation of lung[2] and gastrointestinal enzyme systems[3] and promote hepatic glycogen deposition[4]. Corticotropin-

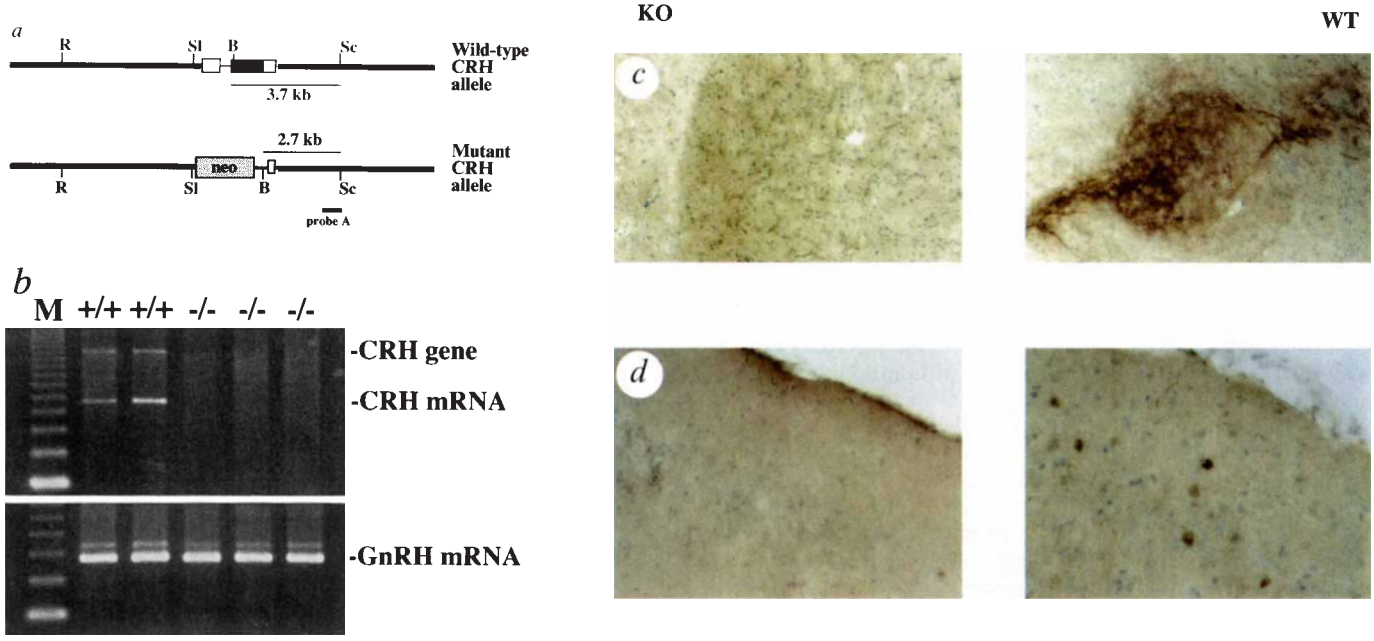


FIG. 1 CRH-deficient mice produced by targeted disruption in embryonic stem cells. *a*, Restriction maps of the normal murine CRH locus and the CRH locus resulting from targeted mutation of embryonic stem cells are shown. Homologous recombination resulted in replacement of the entire pre-proCRH coding region with the neomycin resistance gene[10]. The two exons of the CRH gene are indicated by the boxes on the top line, with the shaded area indicating the pre-proCRH coding region. Restriction sites indicated are *Bam*HI (B), *Eco*RI (R), *Sal*I (SI) and *Sac*I (Sc). Probe A, a 350 bp *Eco*RI–*Sac*I fragment lying outside CRH genomic sequences incorporated in the targeting construct, was used to genotype mice, yielding bands of 3.7 kb for the wild-type allele and 2.7 kb for the CRH-null allele after hybridization to genomic DNA digested with *Bam*HI and *Sac*I. *b*, Reverse transcription-PCR analysis of whole brain RNA from 2 wild-type (+/+) and 3 CRH knockout (−/−) mice at embryonic day 18.5. Total RNA (2 μg) was prepared by the guanidium thiocyanate-caesium chloride method[27] and subjected to reverse transcription from random hexamer primers. Specific cDNAs for CRH, or gonadotropin releasing hormone (GnRH) (as a control for sample integrity), were amplified for 35 cycles using intron-spanning primers as described previously[10]. Amplification products were fractionated through 1.3% agarose and visualized by ethidium bromide staining of gels. The products expected for the wild-type CRH gene (1,267 bp) and mRNA (585 bp) are seen in the wild-type mice, whereas no products are evident in the knockouts. A 123 bp DNA ladder (M) was used as the size marker. c, Immunohistochemical analysis of CRH expression in the central nucleus of the amygdala in knockout (KO) and wild-type (WT) mice. Brains from male wild-type and knockout mice were fixed by perfusion with 4% paraformaldehyde–PBS, followed by immersion in 4% paraformaldehyde for 2 days. Tissue was transferred to 30% sucrose in PBS, cut into 20-μm sections, and reacted with a rabbit polyclonal anti-CRH antiserum[28]. Products were visualized with an ABC Elite peroxidase kit followed by Nissl counterstaining. Abundant CRH immunoreactivity is present in the wild-type section, whereas none is found in the knockout. *d*, Immunohistochemical analysis of CRH expression in the cerebral cortex. Brains were prepared as described in *c* and subjected to immunostaining with the rabbit polyclonal anti-CRH antiserum. Scattered positive neurons are seen in cortical layers II and III, along with diffuse fibre staining traversing these layers in the wild-type animal, whereas no staining is observed in the CRH knockout mouse.

METHODS. Ten to fifteen D3[29] embryonic stem cells which had undergone targeted mutation of one of their CRH alleles[10] were injected into C57BL/6J blastocysts at 3.5 days post-coitum[9]. Chimaeric animals resulting from injected blastocysts were mated to C57BL/6J mice, with germ-line transmission indicated by agouti coat colour in offspring. Heterozygous mice were verified by Southern blot analysis, then mated to produce homozygous CRH-deficient mice.

releasing hormone (CRH), a 41-amino-acid neuropeptide produced in the paraventricular nucleus of the hypothalamus and many regions of the cerebral cortex[5,6], has been implicated in both the HPA axis[7] and behavioural responses[8] to stress. To define the importance of CRH in the response of the HPA axis to stress and fetal development, we have constructed a mammalian model of CRH deficiency by targeted mutation in embryonic stem (ES) cells[9]. We report here that corticotropin-releasing hormone-deficient mice reveal a fetal glucocorticoid requirement for lung maturation. Postnatally, despite marked glucocorticoid deficiency, these mice exhibit normal growth, fertility and longevity, suggesting that the major role of glucocorticoid is during fetal rather than postnatal life.

Mice heterozygous for a null CRH allele generated as shown in Fig. 1*a* are phenotypically normal, and fertile. Mating of heterozygous animals produces viable homozygous CRH-deficient mice at the expected frequency, with 61 (23%) −/−, 131 (49%) +/− and 74 (28%) +/+ in the first 266 mice genotyped at 4 weeks of age. CRH-deficient male and female mice, the oldest of which are now 1 year of age, are equal in size to their littermates and appear healthy without glucocorticoid replacement.

To confirm CRH deficiency, RNA and protein analyses of homozygous mutant and wild-type animals were done. Whole-brain RNA from fetuses at 18.5 days' gestation was subjected to reverse transcription-polymerase chain reaction (RT-PCR) analysis using CRH-specific primers[10]. As shown in Fig. 1*b*, in wild-type (+/+) mice, products are detected from the CRH gene and messenger RNA, whereas in homozygous knockout animals neither product is observed. Immunohistochemical analyses of CRH expression reveals abundant CRH immunoreactivity in wild-type animals, but no staining in the homozygous deficient mice in the central nucleus of the amygdala (Fig. 1*c*) and cerebral cortex (Fig. 1*d*), sites believed important for CRH-mediated behavioural responses. Staining is also absent in the paraventricular nucleus of the hypothalamus in knockout mice (data not shown).

The effect of CRH deficiency on hypothalamic, pituitary and adrenal development was assessed in 8-week-old male mice. No significant structural differences in the hypothalamic paraventricular nucleus, the primary site of synthesis of CRH destined to stimulate the pituitary–adrenal axis, are apparent in knockout and wild-type mice (Fig. 2*a*). Surprisingly, arginine vasopressin (AVP) immunostaining is not increased in the CRH-deficient animals despite low corticosterone levels in basal and stimulated states (see below). Oxytocin immunostaining is also equivalent in the knockout and wild-type male mice (data not shown).

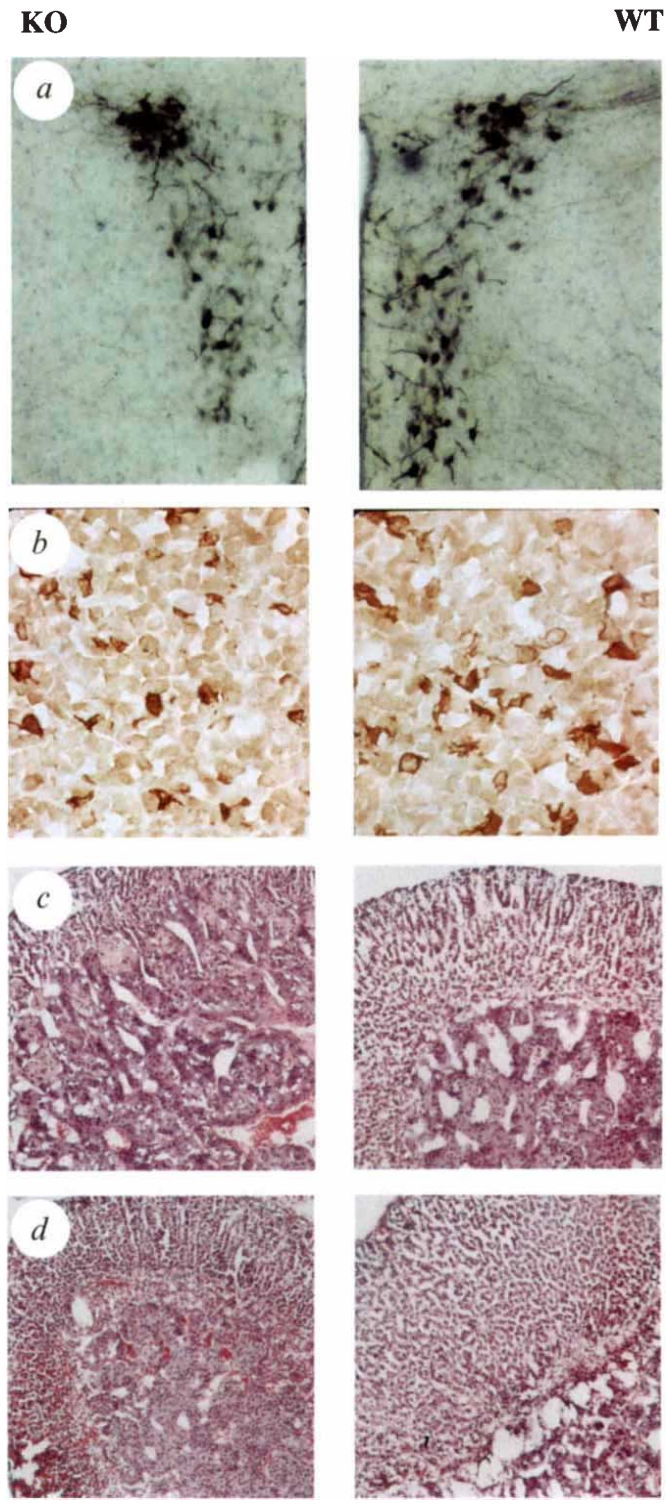


FIG. 2 Immunohistochemical analysis of the HPA axis in CRH knockout mice. *a*, Vasopressin immunostaining in the paraventricular nucleus of the hypothalamus is not increased in CRH knockout mice. Brains from 8-week-old male knockout (KO) and wild-type (WT) mice were fixed as described in Fig. 1c followed by reaction with a polyclonal vasopressin neurophysin antiserum[30]. Antigen–antibody complexes were visualized by peroxidase staining, with anatomy defined by Nissl counterstaining. *b*, ACTH-producing corticotrophs develop in the anterior pituitary gland in the absence of CRH. Pituitaries fixed by 4% paraformaldehyde were cut into 20-μm sections, and reacted with a rabbit polyclonal ACTH antiserum (INCSTAR Corporation, Stillwater, MN). *c*, Adrenal hypoplasia develops in CRH-deficient mice. Adrenals from 8-week-old male knockout and wild-type mice were haematoxylin–eosin stained after paraformaldehyde fixation. The characteristic strand-like appearance of the zona fasciculata is seen in the wild-type adrenal, whereas the knockout has very little zona fasciculata between the adrenal medulla (the medial, inferior region of each section) and zona glomerulosa (located beneath the adrenal capsule). *d*, CRH-deficient female mice also exhibit adrenal zona fasciculata hypoplasia, but less than that of CRH-deficient males.

CRH has been demonstrated to be mitogenic for corticotrophs *in vivo*[11], suggesting a role for CRH in corticotroph development. Evaluation of the anterior pituitary reveals prominent ACTH (or proopiomelanocortin) immunoreactivity both in homozygous CRH-deficient and wild-type animals (Fig. 2*b*). The number and location of positive-staining cells is similar in wild-type and knockout animals, as was immunostaining of the intermediate lobe of the pituitary. Thus, though some hypothalamic releasing factors, such as growth hormone-releasing hormone[12], function as growth or differentiation factors for their target cell type, all need not.

In contrast to the normal appearance of the pituitary is the markedly atrophic appearance of the zona fasciculata of the adrenal gland, the area primarily responsible for corticosterone production in the mouse (Fig. 2*c*, *d*). Especially striking is the more atrophic appearance of the male knockout zona fasciculata in comparison with the female knockout. This is consistent with the decreased adrenal response to stress demonstrated by CRH-deficient mice in Fig. 3. The zona glomerulosa, responsible for mineralocorticoid production, appears histologically normal, consistent with the similar levels of plasma aldosterone in CRH-intact and knockout mice (25.3 ± 6.6 ng dl^{-1} versus 24.2 ± 7.9 ng dl^{-1}, respectively, $P = 0.92$). Adrenal medulla histology also appears normal.

CRH-deficient mice exhibit an impaired, sexually dimorphic adrenal response to stress (Fig. 3*a*). After restraint followed

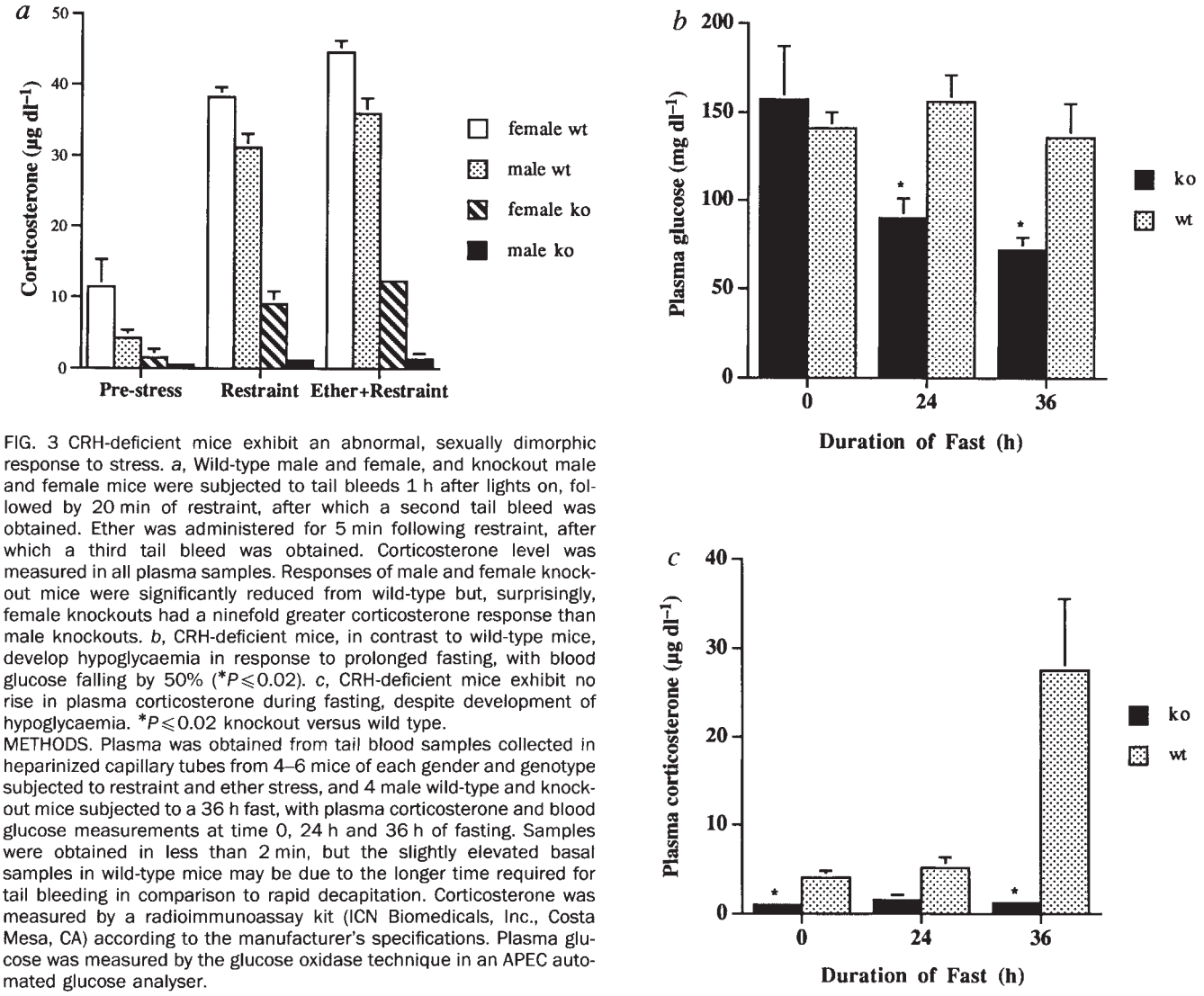


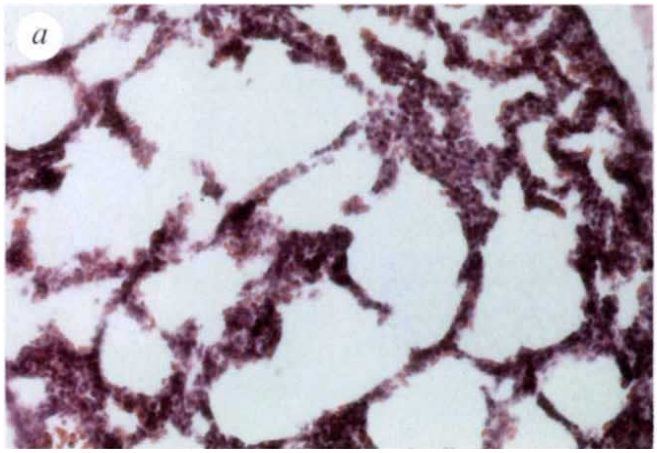
FIG. 3 CRH-deficient mice exhibit an abnormal, sexually dimorphic response to stress. *a*, Wild-type male and female, and knockout male and female mice were subjected to tail bleeds 1 h after lights on, followed by 20 min of restraint, after which a second tail bleed was obtained. Ether was administered for 5 min following restraint, after which a third tail bleed was obtained. Corticosterone level was measured in all plasma samples. Responses of male and female knockout mice were significantly reduced from wild-type but, surprisingly, female knockouts had a ninefold greater corticosterone response than male knockouts. *b*, CRH-deficient mice, in contrast to wild-type mice, develop hypoglycaemia in response to prolonged fasting, with blood glucose falling by 50% (*$P \leqslant 0.02$). *c*, CRH-deficient mice exhibit no rise in plasma corticosterone during fasting, despite development of hypoglycaemia. *$P \leqslant 0.02$ knockout versus wild type.
METHODS. Plasma was obtained from tail blood samples collected in heparinized capillary tubes from 4–6 mice of each gender and genotype subjected to restraint and ether stress, and 4 male wild-type and knockout mice subjected to a 36 h fast, with plasma corticosterone and blood glucose measurements at time 0, 24 h and 36 h of fasting. Samples were obtained in less than 2 min, but the slightly elevated basal samples in wild-type mice may be due to the longer time required for tail bleeding in comparison to rapid decapitation. Corticosterone was measured by a radioimmunoassay kit (ICN Biomedicals, Inc., Costa Mesa, CA) according to the manufacturer's specifications. Plasma glucose was measured by the glucose oxidase technique in an APEC automated glucose analyser.

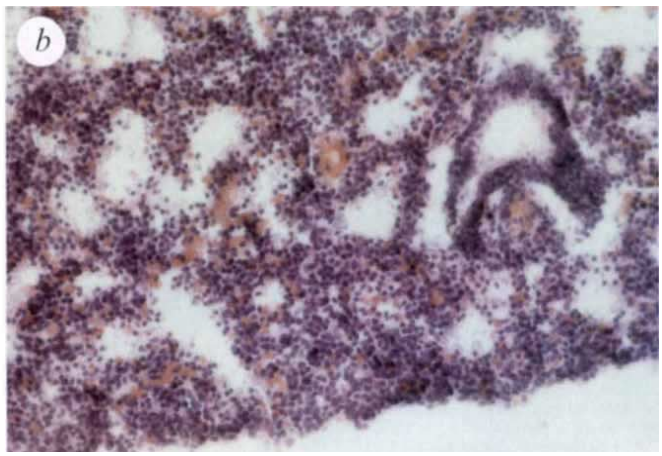
by ether exposure, wild-type female mice achieve corticosterone levels of 44.5 ± 1.7 μg dl^{-1}, whereas knockout female mice achieve levels of only 12.3 ± 0.7 μg dl^{-1} ($P<0.0001$). In wild-type male mice, plasma corticosterone rises to 32.4 ± 0.9 μg dl^{-1} after the combined stress, whereas in male CRH-deficient mice it is only 1.3 ± 0.8 μg dl^{-1} ($P<0.0001$), a value similar to the minimum level observed at the circadian nadir in normal rodents[13,14]. Male heterozygotes exhibit a pattern of response to restraint and ether that does not differ from wild-type males (data not shown).

Because food withdrawal is a potent activator of the HPA axis[15], wild-type and CRH-deficient male mice were subjected to a 36 h fast (Fig. 3*b*, *c*). During the fast, wild-type mice had no fall in plasma glucose and exhibited a significant, sevenfold rise in plasma corticosterone. In contrast, the CRH-deficient mice developed hypoglycaemia by 24 h into the fast. Despite hypoglycaemia, the knockouts had no elevation in corticosterone, which remained <1.5 μg dl^{-1} throughout the fast.

Despite an impaired response to stress, male and female CRH-deficient mice exhibit normal viability and fertility without glucocorticoid replacement. Matings between homozygous CRH-deficient mice result in pregnancies which deliver at 19–20 days' gestation, but yield progeny which all die within the first 12 h of life despite a normal appearance at the time of delivery. Because there is no increase in mortality of CRH-deficient animals from heterozygote matings, the heterozygous mother must provide a factor which crosses the placenta and is capable of rescuing the homozygous offspring. CRH, present in minimal amounts during rodent (in contrast to primate[16]) gestation, is unlikely to cross the placenta in amounts capable of augmenting fetal adrenal activity. To investigate corticosterone deficiency during gestation as the aetiology of fetal demise, corticosterone (30 μg ml^{-1} in the drinking water[17]) was administered to pregnant homozygous female mice which had been mated with homozygous males, beginning at 12 days' gestation and extending through post-partum day 14. This has uniformly resulted in the production of viable litters which have survived without subsequent postnatal glucocorticoid treatment.

Explanation for this effect of perinatal corticosterone on neonatal viability was revealed by histological examination of newborn lungs. Offspring of homozygous matings show lung dysplasia in comparison to wild-type neonates at 2 h of life (Fig. 4*a*, *b*). Marked hypercellularity of the lungs, thickened alveolar septae, and a paucity of air spaces are apparent in knockout animals compared to wild-type. Biochemical evidence of impaired lung function and increased alveolar surface tension, consistent with the abnormal histology, was obtained by analysis of surfactant apoprotein mRNA[2,18]. Surfactant apoprotein-B mRNA, which displays glucocorticoid responsiveness in lung explants[2,19], is reduced to 44% of the wild-type value on embryonic day 18.5 ($P<0.05$). Administration of corticosterone beginning at day 12 of gestation completely reverses the abnormal lung architecture in homozygous deficient mice from homo-





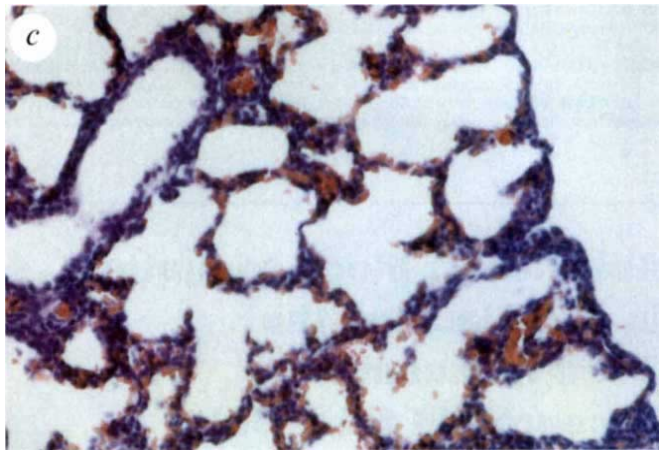


FIG. 4 Lung dysplasia in CRH-deficient offspring of homozygous CRH-deficient matings is rescued by *in utero* corticosterone replacement. *a*, Wild-type lung on day 1 of life demonstrates thin alveolar septae and prominent air space expansion. Lungs were fixed in 4% paraformaldehyde, cut into 20-μm sections and haematoxylin–eosin stained. The area shown is from the lateral region of left lower lobe, as are the following sections from knockout mice. *b*, Lung histology on day 1 of life from offspring of homozygous CRH knockout matings reveals dense, hypercellular lungs, thick alveolar septae and a paucity of air spaces. *c*, *In utero* treatment of offspring of homozygous matings with corticosterone (30 μg ml^{-1}) in the maternal drinking water results in normalization of lung architecture and neonatal viability.

zygous matings (Fig. 4*c*), and increases surfactant apoprotein-B mRNA levels at embryonic day 18.5 such that they do not differ significantly from wild-type controls (78% of wild type, $P=0.14$) but are significantly increased from untreated homozygous knockouts ($P<0.05$).

Pharmacological administration of glucocorticoids to pregnant women has long been a therapy for hyaline membrane disease, with increases in preterm infant viability after maternal administration[20,21,22]. *In vivo* and *in vitro* studies have documented the ability of glucocorticoids to promote lung maturation[2,19,23]. The absolute requirement for glucocorticoid as a component of normal lung development in an intact system had not been rigorously established, however. We show that both maternal and fetal circulations must be glucocorticoid deficient to cause abnormal lung development and decreased fetal viability. This may explain why children with fetal deficiencies in steroidogenesis (congenital adrenal hyperplasia) or congenital adrenal hypoplasia have normal pulmonary development despite very low glucocorticoid production.

CRH-deficient mice exhibit a significantly impaired ability to secrete corticosterone from the adrenal gland in response to stress. This demonstrates that other ACTH secretagogues cannot compensate for the loss of CRH on adrenal development or corticosterone production. One unexpected finding in these studies is the sexual dimorphism in adrenal responsiveness exhibited in CRH-deficient mice. Greater basal and stimulated corticosterone levels in normal female versus male rodents, similar in magnitude to the findings in our wild-type mice, have been demonstrated by several groups[13,24,25]. However, the ninefold higher level of corticosterone following stress achieved by knockout females compared with males implicates a role for sex steroids in regulation of the HPA axis that is not mediated through CRH.

The apparent well being of the CRH-deficient mice, especially the males, contradicts the dogma of glucocorticoids being essential for adult survival. Possibly, the maximum corticosterone levels of ~1–2 μg dl^{-1} that male CRH-deficient animals achieve (at both circadian nadir and peak, our unpublished observation) may fulfill an essential, minimal, permissive glucocorticoid requirement[26], especially as these mice are deficient throughout development and may have chronically accommodated in some way to the low level of glucocorticoid production. Alternatively, high CRH levels in intact animals, rather than low glucocorticoid levels, may confer many of the manifestations characteristic of adrenal insufficiency, such as anorexia, wasting, malaise, fatigue and decreased fertility. Thus, with the possible exception of stimuli more severe than those tested here, the primary importance of glucocorticoid may be in regulating fetal development, rather than supporting survival in adult life. □

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Absence of blood formation in mice lacking the T-cell leukaemia oncoprotein tal-1/SCL

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CHROMOSOMAL translocations associated with malignancies often result in deregulated expression of genes encoding transcription factors[1]. In human T-cell leukaemias such regulators belong to diverse protein families and may normally be expressed widely (for example, Ttg-1/rbtn1, Ttg-2/rbtn2)[2,3], exclusively outside the haematopoietic system (for example, Hox11)[4], or specifically in haematopoietic cells and other selected sites (for example, tal-1/SCL, lyl-1)[5,6]. Aberrant expression within T cells is thought to interfere with programmes of normal maturation. The most frequently activated gene in acute T-cell leukaemias, *tal-1* (also called SCL)[7,8], encodes a candidate regulator of haematopoietic development[9], a basic-helix–loop–helix protein[5], related to critical myogenic[10] and neurogenic[11] factors. Here we show by targeted gene disruption in mice[12] that *tal-1* is essential for embryonic blood formation *in vivo*. With respect to embryonic erythropoiesis, *tal-1* deficiency resembles loss of the erythroid transcription factor GATA-1[13,14] or the LIM protein rbtn2[15]. Profound reduction in myeloid cells cultured *in vivo* from *tal-1* null yolk sacs suggests a broader defect manifest at the myelo-erythroid or multipotential progenitor cell level.

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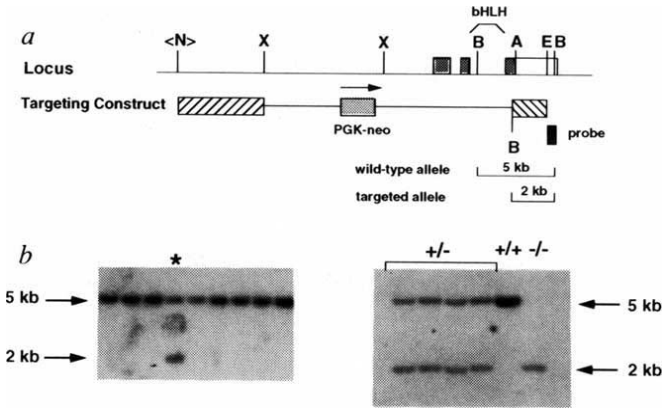

FIG. 1 Targeted disruption of the mouse *tal-1* locus. *a*, Partial restriction map of the mouse *tal-1* locus with the targeting construct depicted below. Homologous recombination resulted in the replacement of all genomic sequences lying between the two regions of homology (hatched boxes) by the PGK-*neo* cassette. X, *Xho*I; B, *Bam*HI; A, Asp718; E, *Eco*RI; bHLH, basic-helix–loop–helix region; ⟨N⟩, an artificial *Not*I site within the polylinker of the λFixII phage vector. The three terminal exons of the gene are indicated as closed boxes in top diagram. The bHLH region is encoded by the two indicated exons. The open box represents the 3′-untranslated region. A herpes virus-thymidine kinase cassette was present in the pPNT[24] cloning vector, outside the indicated region of homology. The wild-type (5 kb) and targeted (2 kb) *Bam*HI fragments, as detected by a probe at the extreme 3′-end of the gene, are indicated below. *b*, Disruption of the *tal*-1 locus. Left, Southern blot analysis of ES cell clones. A targeted clone is indicated by the asterisk. Right, Southern blot analysis of E10.5 embryos resulting from interbreeding of *tal-1*$^{+/-}$ mice.

METHODS. Overlapping genomic phage clones of the *tal-1* locus were isolated from a λFixII strain 129 library (Strategene) by standard procedures[25]. Partial restriction mapping, PCR analysis, and limited DNA sequencing were done to identify exons, particularly those encoding the bHLH domain[26]. The 5-kb 5′- (⟨N⟩−X) and 2-kb 3′- (A-E) fragments were cloned into the vector pPNT[24] to generate the targeting construct. The 3′-flanking probe was generated by PCR labelling using a specific primer pair (5′-TGTTCTCATACTCCATACCCC-3′/5′-AAGTACGGCTAGACCC-ACCAA-3′) and a 3′-untranslated region fragment as template. The targeting construct was linearized with *Not*I and electroporated into J1 ES cells as described[27,28]. Clones were selected in G418 (250 μg ml^{-1}) and ganciclovir (2.5 μM), expanded and subjected to Southern blot analysis using the 3′-probe. Homologous recombination was observed at a frequency of ~1/200 G418/ganciclovir-resistant clones. Chimaeras were generated by injection of C57BL/6 blastocysts as previously described[28]. Genotyping of liveborn offspring and embryos was done by either Southern blot or PCR analysis.

To disrupt the *tal-1* gene, we constructed a targeting vector in which a neomycin-resistance cassette replaced coding sequences (Fig. 1*a*). Homologous recombination in transfected embryonic stem (ES) cells was detected by Southern blot analysis (Fig. 1*b*). Heterozygous mice (*tal-1*$^{+/-}$) appeared normal and were bred together. Among 76 pups from 12 litters no liveborn *tal-1*$^{-/-}$ mice were observed. At embryonic day 8.5 (E8.5) ~25% of viable embryos were of the *tal-1*$^{-/-}$ genotype; none was identified after E10.5. Thus, loss of *tal-1* is lethal between E8.5 and E10.5. The *tal-1*$^{-/-}$ embryos lacked genomic sequences represented in *tal-1* messenger RNA (data not shown).

E9.5–10 *tal-1*$^{-/-}$ embryos were readily distinguished from littermates by their smaller size, profound pallor, and a dilated pericardial sac (Fig. 2*a–c*). Although overall growth of *tal-1*$^{-/-}$ embryos was retarded, major developmental milestones, including chorio-allantoic fusion, rotation of the embryo, formation of head structures, closure of the caudal neural pore, and initiation of cardiac contractions, were attained. Further visual inspection revealed that *tal-1*$^{-/-}$ embryos were bloodless. Histological analysis established the absence of embryonic (nucleated) red cells in the embryo, yolk sac and placenta but demonstrated