Results and discussion. The Figure shows the results of radioimmunoassays (A) and bioassays (B) of LH released from fetal mouse pituitaries into the incubation media, with or without LRF. The LH release measured by RIA was clearly stimulated by addition of 5 or 50 ng LRF to the incubation system. The decrease in LH content following removal of LRF was significant in medium III ($p<0.02$), but not in medium V, and did not reach the control level. The high LH level in these media as compared with the control medium (I) may be due to the presence of LRF remaining in the incubation flask and/or to a long-term stimulating effect of LRF upon these pituitaries.

It is noteworthy that the LH stimulation after 50 ng LRF (media IV) was not greater than after 5 ng (media II). This might indicate that a maximal response is obtained by addition of 5 ng LRF. However, an exhaustion phenomenon cannot be excluded.

The bioassays of LH are in good agreement with RIA. This reinforces the reliability of our present and previous results[14]. Moreover, the highly significant increase in biologically assayable LH under LRF suggests that hypothalamic stimulation of fetal pituitary may produce a detectable effect on the fetal testis. There is good evidence in humans that the biological activity of LH arizes from its β-subunit which seems to be synthetized under hypothalamic influence[15,16]. If this is the case in mouse, we could tentatively speculate that the hypothalamo-gonadotropin axis is already functional in mouse at the end of intra-uterine life, as it was suggested for other pituitary hormones, including TSH, GH and ACTH[17]. At least we can conclude that mouse fetal pituitary is responsive to LRF.

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Effects of Injury on the Concentration of α_1-Macroglobulin and α_2-Macroglobulin in the Plasmas of Male and Female Rats

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Summary. The effects of injury on the concentration of α_1-macroglobulin and α_2-macroglobulin in the plasmas of male and female rats has been investigated. At 5 days after injury to the male rats the α_1-macroglobulin concentration increased to 131% of its preinjury value. The α_2-macroglobulin concentration increased more rapidly to a maximum of 86 times its initial value. In the female rats α_2-macroglobulin increased only slightly and α_1-macroglobulin not at all.

The two rather similar α-macroglobulins of the rat have been referred to as: α_1-macroglobulin (α_1M), or slow α_1-globulin[3]; α_2-macroglobulin (α_2M), or slow α_2-globulin[4]; α_2-glycoprotein (GP)[5]; α_2 (acute phase)-globulin[6]; α-2-GP[7].

An increased concentration of α_2M in the plasmas of rats has often been employed as an index of tissue damage. Although present at high concentration in foetal rats α_2M is present at less than 50 μg/ml in the plasmas of normal male laboratory rats[8]. After injury its concentration may rise as much as 40 times. However after adrenalectomy injury no longer leads to this increase[9,10]. During recent work aimed at purification of rat α_2M lower concentrations of this protein were found in the plasmas of injured female as compared to injured male rats of the same strain. In order to confirm and extend this finding the concentration of α_2M in the plasmas of male and female rats has been estimated at various times after injury. For purposes of comparison the concentration of α_1M in the same plasmas was also estimated.

In a separate experiment the plasma haptoglobin concentration of injured male and female rats was estimated.

Materials and methods. Male and female hooded rats of the strain maintained at NIMR (weighing 170–190 g) were used throughout the experiments. All the rats received an i.m. injection of 2.5 mg cortisone (Cortisone Acetate Injection BP) into a hind thigh and 0.4 ml purified turpentine oil s.c. divided equally between both flanks, and then after 1–12 days the various groups were bled. The injections and bleedings which were by cardiac puncture under light anaesthesia were always done between 14.00 and 15.00 h. After storage of the sera for up to 7 days at 2 °C, the α_1M and α_2M concentrations were measured by radial immunodiffusion[11] using monospecific rabbit antisera against α_1M and α_2M and purified samples of the two proteins as standards. When very low concentrations of α_2M were to be estimated, the very faint rings were intensified by soaking the gel in 0.5% phosphotungstic acid. Haptoglobin was estimated by its peroxidase activity when complexed with methaemoglobin[12]. All values in the text are expressed as means $\pm$ SD.

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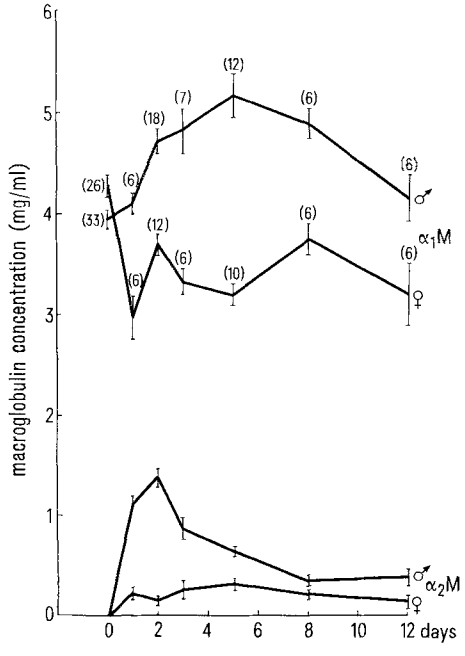
Results. As shown in the Figure injection of turpentine and cortisone led to greatly increased concentration of α_2M in the plasmas of male rats. Thus starting at only 0.016 ± 0.001 mg/ml α_2M rose at 2 days to a maximum of 1.38 ± 0.35 mg/ml. It was still well above normal at 12 days. In sharp contrast the same injury to female rats led to much smaller and more variable responses. Thus out of 18 female rats investigated at 2 and 3 days after injury 10 averaged only 0.049 ± 0.024 mg/ml whereas the other 8 gave an average of 0.278 ± 0.098 mg/ml. The average control value for non-injured female rats was 0.01 ± 0.005 mg/ml. It is possible that the degree to which female rats respond to injury is related to the various stages of the oestrus cycle.

Before injury the major α-macroglobulin, α_1M, was present at a slightly higher concentration in the plasmas of female compared to that of male rats ($p < 0.01$). Following injury there was an increase in concentration of this protein of 31% at 5 days in the plasmas of the males. In the females the α_1M concentration declined by 23% to a level which was 61% of that of the males when the α_1M values of the latter were at their peak. Subsequently the concentration of the α_1M in the plasmas of both the male and female rats fell, returning in the case of the males to the preinjury concentration at 12 days.

Haptoglobin concentrations were estimated in plasmas from 6 uninjured male and 6 uninjured female rats and also in similar-sized groups at 1 day after injection of cortisone and turpentine. A maximum increase of haptoglobin concentration in the blood of female rats at this time after turpentine injection has already been reported[13]. The average haptoglobin concentration in the plasma of uninjured males was 1.66 ± 0.46 mg/ml while that in females was 0.59 ± 0.25 mg/ml. The higher basal value in males agrees with similar observations in humans[14]. One day after injury, however, the haptoglobin concentrations of both male and female rats increased to approximately equal values (5.72 ± 0.11 for males, 5.06 ± 0.57 for females).



Changes in concentration of α_1-macroglobulin and α_2-macroglobulin after injection of turpentine and cortisone into male and female rats as means ± SEM. The numbers of rats employed for each time point are given in parentheses.

Discussion. All the rats received both i.m. injection of cortisone and s.c. injection of turpentine. Cortisone was used because α_2M synthesis is known to be corticosteroid dependent[9,10]. A further reason was that preliminary experiments had suggested that excess cortisone would increase the rates of synthesis of this protein at all times after injury. Because of this aspect of the experiment it is important to emphasize that all the results relate to rats receiving excess cortisone rather than to normal rats.

As shown in the Figure the concentrations of both α_1M and α_2M increased in the plasmas of the male rats after injury. In the females on the other hand the concentration of α_2M increased only slightly whereas that of α_1M fell.

The increase in concentration of α_1M which occurred after injury in the plasmas of the male rats means that this protein, at least in males, must be considered to be an 'acute phase reactant' (AP-reactant)[15]. As mentioned above no such increase of α_1M occurred in the females. It is notable however that in female rats suffering from experimental immune complex nephritis, Horne and Toner[16] observed that increased concentrations of α_1M occurred after 8 weeks. Male rats were not investigated. The present findings would appear to be the first report of an 'acute phase' response by a particular protein occurring in only one sex.

The synthesis of both proteins appears to be regulated similarly, with that of α_2M being the more sensitive to the stimulus of injury. Thus injury in males causes a marked increase in α_2M and only a minor one in females, whereas under the same conditions α_1M responds only to a small degree in males and not at all in females. The difference in response between males and females may be due to qualitative and quantitative differences in their sex hormones. That a sex dependent response to injury is not a general phenomenon for acute phase proteins is shown by the results obtained for haptoglobin. In this case both sexes showed an equal response to injury.

α_2M is known[4] to increase in concentration during pregnancy in both foetal and maternal plasma with very high levels persisting in the new-born. High α_1M concentrations occur perinatally in maternal rat plasma[3]. The same authors have also shown that α_1M attains high concentrations in males as compared with females after the age of 13 weeks. Because at this age the male rat begins to produce androgens, the possibility that these hormones may stimulate the rate of synthesis of α_1M should be considered. Taken altogether the evidence now available strongly suggests that in rats sex hormones are involved in the synthesis of both α_1M and α_2M. One of the functions of these hormones would seem to be to modulate the stimulatory response brought about by injury.

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