Journal of Steroid Biochemistry, 1977, Vol. 8, p. 577. Pergamon Press. Printed in Great Britain

GENERAL DISCUSSION

Bloch. Concerning Dr. Silman's communication, I should like to show you some results obtained with Professor Bugnon, dealing with the immunocytological detection of corticotropic cells in the human fetal pituitary (ref. [9, 10, 11, 16] in text). We used various antisera directed against ACTH or MSH. We used anti 1–24 ACTH, anti 17–39 ACTH, anti β MSH and anti α MSH.

ANTIGENS / I.S.	(1–24) ACTH 250 µg/ml	(17–39) ACTH 300 µg/ml	(25–39) ACTH 300 µg/ml	α MSH 300 µg/ml
anti (1–24) ACTH	−	−	+	+
anti (17–39) ACTH	+	−	−	+
anti α MSH	+	+	+	−
anti β MSH	+	+	+	+

− = total inhibition of immunocytological reactions.
+ = no inhibition of immunocytological reactions.

Immunocytological tests of inhibition in various species from fish to man showed that anti 1–24 ACTH is directed against the 17–24 portion of the ACTH, that anti 17–39 ACTH is directed against 25–39 ACTH, that anti β MSH does not cross react with ACTH neither does anti α MSH that is only directed against α MSH. [Moreover, in the rat or in the tortoise, anti α MSH and anti β MSH did not reveal corticotropic cells in the anterior pituitary but they both revealed the pars intermedia cells of these two species.]

If glycoprotein hormone containing cells are few in number at the early stages of fetal life as described before, corticotropic cells are very numerous and they are indifferently stained by anti 1–24 ACTH, 17–39 ACTH, and by anti β MSH but they are never revealed by anti α MSH immune serum. So we can say that from the 8th week of fetal life, the pituitary contains ACTH and β MSH. But we never found any α MSH; we did not study the late stage of fetal life near birth, when you found α MSH. Dubois *et al.* have found α MSH in the human adult pituitary (*Bull. Ass. Anat.*, **57** (1973) 63–76).

Naftolin. What portion of the gland?

Bloch. Here it is only the anterior lobe but you can see the beginning of the caudal wall of the hypophyseal pouch where we found some stained cells. At the 15th week of fetal life we found, with anti 17–39 ACTH, that corticotropic cells are stained in the anterior pituitary and also behind the pituitary cleft.

Silman. The failure to find α MSH is of course a problem between you and Dr. Swaab since he has presented cytoimmunological evidence for its presence. As far as our work is concerned with the human fetal pituitary, we purposely did not employ a specific α MSH assay since we were performing chromatography and did not want to prejudge the issue as to what sort of *N*-terminal ACTH fragment we might find. If you really have a totally specific α MSH antibody then it is probably binding to the acetylated or amidated end groups. All we claim to have found is an α MSH-like peptide, and though its biological potency is far greater than ACTH it is only about 5% that of synthetic α MSH. Interestingly, your 17–39 antisera stains cells in the pars intermedia and though this could represent intact ACTH, it could also be something like CLIP.

Jaffe. Have you ruled out CLIP as a contaminant?

Bloch. We think that anti α MSH is very specific for α MSH. As a matter of fact, in addition to the inhibiting tests that we carried out we have verified that in various species (fishes, tortoise, rat, cat and fox) it only reveals the pars intermedia without any staining of the anterior pituitary. Anti 17–39 ACTH is directed against 25–39 ACTH. So it can reveal the entire molecule of ACTH or only CLIP. In human adenoma, Tramu *et al.* (*Ann. Endocr.* **37** (1976) 55–56) have found that corticotropic cells could be stained by anti 17–39 ACTH only. In some Teleost fishes (*Boops salpa*), Bugnon and Fellmann in collaboration with Malo-Michele (*C. R. hebd. Séane. Acad. Sci., Serie D*, **283** (1976) 643–646) have demonstrated that some corticotropic cells of the proadenopituitary could be stained in some particular conditions by anti 17–39 ACTH but not by anti 1–24 ACTH.

Dörner. Japanese colleagues (Daikoku *et al.*) have recently published some papers on the differentiation of rat pituitary explants in different stages of prenatal life. In these studies, they found a clear cut differentiation of the anterior pituitary without any contact with the hypothalamus. Would you think that there are species-specific differences regarding the autonomous differentiation of the hypophysis?

Bloch. In the human fetus, few data are available. Pasteels *et al.* (ref. [32] in text) demonstrated that *in vitro* LH RH could stimulate the liberation of LH as early as the 13th week of fetal life. Tamura *et al.* (ref. [34] in text) has shown that LH and FSH could be released *in vitro* from the 16th week under the action of LH RH. Dubois *et al.* (*Ann. Endocr.* **36** (1975) 321) found only α subunits in gonadotropic cells of an anencephalic newborn. This last work suggested that hypothalamus was necessary in the human for the complete differentiation of the gonadotropic cells able to synthetize β subunits.

Naftolin. Would you or Dr. Silman like to talk a little bit about CRF and how CRF might work? Is it possible that it might work initiating some of these cleavages which result in a releasable form of hormone?

Bloch. When cells are active and secrete hormones, they generally show the morphological aspect of differentiated cells with secretion granules. If it is possible to obtain such a differentiation *in vitro* by action of stimulating substance or by medium composition, it is difficult to ascertain that these cells were not predetermined by induction, though they are not morphologically differentiated.

Silman. We have got very little information on CRF. Dr. Lowry in the Department of Chemical Pathology at Barts is working on this problem and thinks that it is a molecule similar to vasopressin. Interestingly, the *N*-terminal penta- or hexa-peptide of oxytocin is said to be an α MSH release-inhibiting factor. This work has been reported by Dr. Celis and others. So what interests us is the relation of all this to the apparent switch in peptide synthesis that we have noted at term in the human fetal pituitary. If a fetal oxytocin-like molecule were to turn off an α MSH-like molecule and turn on, instead, ACTH; and if this led to the turning off of DHES and the turning on of fetal cortisol, then it would seem to bring together some of the apparently unrelated yet outstanding problems of human parturition.

Jaffe. Dr. Tadaki, was that in human fetuses at mid gestation and how were the challenges with the releasing factor performed in the 2nd trimester and at term. By vein or by injection.

Naftolin. By vein, umbilical vein.