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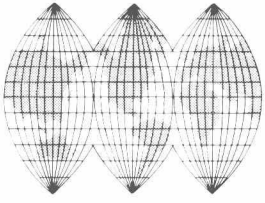

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CONTENTS

Session C 1

Neuroendocrinology and Reproduction

(Abstracts 1-11)

1. Short-term steroid treatment on plasma LH and FSH in castrated rats: from birth to puberty

S. R. OJEDA and V. D. RAMIREZ, *Institute of Physiology, Universidad Austral de Chile, Valdivia, Chile*

Rats were gonadectomized at 5 day intervals from birth to 40 days of age (females) or 55 days (males) and sacrificed 5 days after operation. Following castration females were daily injected subcutaneously with estradiol benzoate (EB) (0.05 μg/100 g body weight) and males with testosterone propionate (TP) (40 μg/100 g). Controls received oil. At sacrifice LH and FSH were determined by radioimmunoassay. The weights of uterus, ventral prostate and seminal vesicles were recorded. EB treatment does not lower plasma FSH levels before 20 days of age, and then it becomes increasingly effective up to 35 days of age. However, at 40 days EB again appears ineffective and at 45 days it partially recovers its effect. With the EB treatment plasma LH levels in ovariectomized rats show approximately the same pattern as FSH. In orchidectomized males TP treatment significantly lowered plasma FSH and LH levels at any age. The responsiveness of the uterus and seminal vesicles to treatment with EB and TP, respectively, increased significantly ($p < 0.01$) when animals reached puberty. It appears that the immature gonadotropin system comes under gonadal control rather later in the female than in the male and also that its sensitivity changes at puberty.

2. Accumulation of brain ^{3}H-catechols in rats given ^{3}H-tyrosine at different phases of the estrous cycle

L. L. ZSCHAECK and R. J. WURTMAN, *Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass., U.S.A.*

We have examined the relationships between the vaginal estrous cycle and the accumulation of ^{3}H-catechols in the brains of rats given ^{3}H-tyrosine i.p. Animals received the ^{3}H-tyrosine between 3 and 6 p.m., and were killed after 10 minutes. The ratio of the specific activities of brain ^{3}H-catechols to ^{3}H-tyrosine rose linearly during this interval and was taken as an estimate of ^{3}H-catechol biosynthesis. This ratio was lowest (0.27) during diestrus, and rose to 1.24 during proestrus. Acute doses of estradiol in aqueous solution (0.22 mg/rat), administered during diestrus, failed to influence this ratio. It is not presently known whether the changes in brain ^{3}H-catechol accumulation that occur during the estrous cycle result from actions of circulating hormones on the brain or from neural events.

3. Effect of testosterone and of anti-androgens on the behaviour of hypothalamic neurons in male rats

P. SABA, V. MARESCOTTI and F. TRONCHETTI, *II Medical Clinic, University of Pisa, Pisa, Italy*

The frequency characteristics of the neuronal discharges in the hypothalamus were recorded, using stereotaxically oriented steel micro-electrodes in adult male rats. In order to prevent EEG variations during the experiment the neuronal activity was recorded after mid-brain transection performed at pontine level, under light urethane anesthesia. In such conditions a wide range of firing rates in hypothalamic neurones was seen though most of the units that were studied had mean rates of 0.2-10/sec. The firing pattern in a single unit was generally regular in time. The infusion of up to 40 μg of testosterone acetate in ethanol (0.1 ml) caused dramatic changes in the spike activity of many units only at posterior hypothalamic levels. No significant variations in EEG pattern were observed during the injection. The first change in spike activity after testosterone administration was usually seen within 3-4 min and the inhibitory effect lasted 20-60 min in proportion to the doses employed. Cyproterone acetate

(5 mg) in 0.2 ml ethanol injected i.v. had no effect on spontaneous activity of hypothalamic neurones but prevented the inhibitory effect of testosterone administered i.v. 10-20 min later. The results seem to suggest that testosterone has a selective action on posterior hypothalamus and further confirm that these neural structures are involved in the control of testicular activity.

4. Adrenal progesterone: control of secretion

F. PIVA, P. GAGLIANO and M. MOTTA, *Department of Pharmacology, University of Milan, Milan, Italy*

Progesterone (P) of adrenal origin may participate in facilitating the release of LH and mating behavior. P measured by a protein binding and B by a fluorometric technique were assayed in the plasma of the following groups of rats: (1) castrated (Cx), (2) Cx-dexamethasone (D) treated, (3) Cx-D-treated given i.v. LH, FSH, prolactin or ACTH. In agreement with previous findings, plasma P never dropped to undetectable levels; a peak was observed 15 days after castration (3.86 ± 0.33 ng/ml). B levels were increased in Cx rats, a peak appearing again at 15 days (35.9 ± 4.57 μg/100 ml). 25 μg/100 g body wt. of D reduced both P and B levels ($P = 2.00 \pm 0.22$, < 0.0005; $B = 1.89 \pm 0.42$, < 0.0005) in 15-day Cx rats. Ovine LH and FSH (10 μg/100 g body wt.) did not stimulate adrenal P and B secretion in D-blocked rats; on the contrary, prolactin (10 U/100 g body wt.) induced a significant increase in P concentrations (6.46 ± 1.41, < 0.0005) without affecting B. ACTH (5 mU/100 g body wt.) brought about a significant increase of both P and B ($P = 3.76 \pm 0.30$, < 0.0005; $B = 35.3 \pm 2.19 < 0.0005$). A purified preparation of human LH (5 μg/100 g body wt.) enhanced plasma P (3.38 ± 0.43, < 0.0025), but left B levels unmodified. It appears that the secretion of adrenal P is controlled by factors different from those which regulate the release of B. ACTH affects both steroids, while prolactin, and possibly LH, stimulate only P, a result which indicates that these hormones do not produce the effect because of contamination with ACTH. (Supported by a grant from the Ford Foundation.)

5. Neutral interaction of ACTH-adrenal rhythm and ovulatory cycles in the rat

N. HAGINO and S. YAMAOKA, *Section of Neurophysiology, Southwest Foundation for Research and Education, San Antonio, Texas, U.S.A.*

Local injection of dexamethasone into the medial preoptic area blocks both ovulation and ACTH secretion, and injection into the septum alters the rhythm of ACTH secretion (14.2 μg/ml vs 9.0 at 8 a.m., 19.6 vs. 23.4 at 3 p.m.) and disrupts the vaginal cycle, blocking ovulation. Under urethane anesthesia, electrochemical stimulation of dorsal hippocampus (DH) principally caused excitation of the activity of septal neurons (extracellular recording; 21 neurons increased, 13 decreased, 14 unchanged) and this stimulus tends to increase plasma corticosterone (50 μg/ml vs 26.8). However, under stressful conditions, this stimulus decreases plasma corticosterone (143 μg/ml vs 175). Parenteral injection of dexamethasone reduced the spontaneous activity of septal neurons and altered the response of these neurons to DH stimulation. Anterior amygdaloid stimulus resulted mainly in inhibition of septal neurons (10 decreased, 4 increased, 3 unchanged). Anteroventral thalamic stimulus tends to decrease the activity of septal neurons (9 decreased, 2 increased, 1 unchanged). It is inferred that the septum is one of the interacting centers for information from either the limbic system or the reticulo-thalamo-cortical system, and regulates the ACTH-adrenal rhythm and ovulatory cycles via the hypothalamus.

6. A possible role for the perinatal gonadotropins in sexual differentiation of the brain

P. J. SHERIDAN*, M. X. ZARROW, B. D. GOLDMAN and V. H. DENENBERG, *Department of Biobehavioral Sciences, University of Connecticut, Storrs, Conn., U.S.A.*

Two preliminary experiments were carried out to determine whether the high levels of serum gonadotropins found in the neonatal female rat play a role in the sexual differentiation of the neuroendocrine axis. Neonatal female rats were injected either with 100 μg of testosterone propionate (TP) alone or with the combination of the TP and gonadotropins (50 μg of FSH and 50 μg of LH/day for 3 days). The concomitant injection of gonadotropins

with TP significantly delayed or prevented the onset of persistent vaginal estrus ($p < 0.01$); and significantly ameliorated both the loss of female sexual behavior ($p < 0.05$) and the decrease in ovarian weight due to the injection of TP ($p < 0.05$). The possible sites of action for the gonadotropins (CNS vs. ovary) are discussed and the hypothesis is presented that the gonadotropins may act directly on the CNS in the sexual differentiation of the neuroendocrine axis. (We thank the NIAMD Pituitary and Rat Pituitary Hormone Distribution Program for the ovine LH and the rat FSH, respectively.)

* NIH predoctoral fellow 5 701 MH 44669-03.

7. Estrogen effects on regional brain blood flow in conscious female rats

E. B. SKELLEY and H. GOLDMAN, *Department of Pharmacology, The Ohio State University, Columbus, Ohio, U.S.A.*

This study was designed to examine the effects of chronic and acute estrogen treatments on regional brain blood flow using the cardiac output fraction method. Cardiac output values, derived from ^{14}C-thiopental in sequential arterial blood samples and the uptake of this isotope in ten brain areas were measured simultaneously in each animal. Comparisons were made among five groups of female Wistar rats including intact controls, ovariectomized controls, intact and ovariectomized animals acutely injected with estradiol and animals in persistent estrus due to neonatal androgenization. Blood flow values were significantly altered in certain brain regions in hormonally treated animals compared to controls. Increased flow was found in the pons, medulla and cerebellum after acute estrogen treatment. Chronically estrogenized (persistent estrus) animals had greater flow in the olfactory bulbs, basal ganglia and midbrain. Both acute and chronic estrogen treatments produced higher flow in the hypothalamus, hippocampus and frontal cortex. The results show differences in effects of chronic and acute effects of estrogen upon regional brain blood flow and demonstrate the use of this parameter in examining the actions of hormones in the brain.

8. Role of the ovulatory gonadotropin surge in the induction of sexual receptivity in the hamster

B. D. GOLDMAN and P. J. SHERIDAN, *Department of Biobehavioral Sciences, University of Connecticut, Storrs, Conn., U.S.A.*

The present experiments were undertaken to study the nature of the relationship (i.e. causal?) between ovulatory gonadotropin (GTH) release and subsequent sexual behavior in the hamster. Female hamsters were injected with GTH antiserum (A/S) or normal rabbit serum (NRS) on the afternoon of proestrus and were tested for sexual behavior during the evening of the same day. A/S-treated animals did not display sexual behavior (0/4) and failed to ovulate; NRS-treated controls displayed lordosis (7/7) and ovulated. When A/S treatment was followed by injection of 50-200 μg ovine LH both ovulation (10/10) and behavior (8/10) were restored. The same effects were obtained with 200 μg ovine FSH; however, 200 μg rat FSH restored ovulation (7/7) but *not* behavior (2/7). A similar effect was seen with 100 μg ovine FSH (0/6 showed lordosis; 9/9 ovulated). Progesterone treatment (200 μg) restored behavior (12/14) but not ovulation (3/14) after A/S. Estradiol benzoate (8-50 μg) was comparatively ineffective in restoring either ovulation (2/6) or behavior (2/12). We conclude that ovulatory GTH release (especially LH) is a necessary event in the chain leading to sexual behavior and that GTH release probably acts via stimulation of progesterone secretion.

9. Sexual maturation in olfactomized female rats

E. W. HALLER, N. SATO, R. D. POWELL and R. I. HENKIN, *University of Minnesota School of Medicine, Duluth, Minn., U.S.A.*

Timing of vaginal opening was measured in 140 female Holtzman rats: in 51, after removal of their olfactory bulbs (olfactomy) at 6 days of age; in 9, after olfactomy at 20 days of age; in 20, after sham operation at 6 days of age; in 60 which served as controls. Body weight, uterine, ovarian and pituitary weights and serum and pituitary LH concentrations were also

measured in rats from each group at varying time periods from 14 to 63 days of age. In rats olfactomized at 6 days of age vaginal opening was significantly delayed by approximately 10 days (olfactomized opening 49.3±2.0 days, mean ±1 S.E. control opening 39.0±0.6 days) but was not delayed in rats olfactomized at 20 days of age or in sham operated controls. In rats olfactomized at 6 days of age uterine and ovarian growth lagged that of controls by approximately 10 days and the phasic variation in pituitary LH content normally present prior to vaginal opening did not occur. Corpora lutea were not present in rats olfactomized at 6 days of age in whom vaginal opening was delayed, but once opening occurred ovarian histology and estrus cycle behavior could not be distinguished from that of controls. These phenomena indicate that olfactory stimuli, acting through the olfactory bulbs, normally influence function of the hypothalamic-pituitary-gonadal axis and that the presence of these influences early in life is critical for normal timing of sexual maturation.

10. Neonatal androgenization and aggression in the male golden hamster

A. P. PAYNE and H. H. SWANSON, *Department of Anatomy, The Medical School, Birmingham, U.K.*

While in most species males show more aggression than females, the golden hamster is anomalous. Both sexes fight, females are dominant over males, and male aggression towards females can be increased by ovarian steroids, but not by androgens. Litters received either 300 μg testosterone propionate (TP) on the day of birth, or oil vehicle alone. At 100-120 days, all males were observed for a ten minute test period on three successive days with an intact dioestrous female matched for body weight. Control males showed significantly less aggression than females and were submissive to them. Conversely, the TP-treated males showed comparable levels of aggression to females and tended to be dominant. Using the same test procedures, castration resulted in both groups being submissive to females, but subsequent treatment with TP (1,000 μg/day) increased aggression in the androgenized males, but not in the controls. It is considered, on the basis of this and other work, that the male golden hamster has an impaired programming of neural structures during the hypothesized critical period of brain differentiation and is therefore of considerable interest in comparative studies with other rodents.

11. Regulation of hormonally mediated maternal nest structure in the mouse by neonatal hormone manipulation

R. D. LISK and J. A. RUSSELL, *Department of Biology, Princeton University, Princeton, N.J., U.S.A.*

Our earlier studies showed that building of the enclosed cocoon-like (maternal) nest is a sexually dimorphic response pattern which is regulated by estrogen plus progesterone. Nest building can be quantified and evaluated through daily measurement of the amount of hay utilized plus classification of the nest by style. Increasing estrogen levels block nest building in both females and males. When low levels of estrogen are given to females addition of progesterone results in both increased nest weight (50 g vs 20 g) and construction of the maternal nest. Males do not show this response. Following neonatal (Day 1) injection of testosterone propionate to the female and injection of estrogen and progesterone in adulthood no maternal nest building response is seen. Males castrated on Day 1 built the maternal nest in adulthood following injections of estrogen plus progesterone. Thus the hormonally elicited maternal nest style becomes sex limited due to exposure of the neonatal animal to androgens. This suggests that differentiation of hormonally mediated nest building is under similar control to those systems mediating sexual responsiveness and ovulation, both of which can be suppressed by exposure to androgen during a sensitive stage in development.

Session C 2

Corticosteroids – Mechanism of Action – Metabolism

(Abstracts 12-21)

12. Alterations in phenylalanine tRNA induced by glucocorticoids in hepatoma tissue culture cells

M. LIPPMAN, S. YANG and E. B. THOMPSON, *National Institutes of Health, Bethesda, Md., U.S.A.*

We have previously shown an increase in phenylalanine acceptor activity in vivo in hepatoma tissue culture (HTC) cells treated with dexamethasone. We have now investigated the kinetics of this response; the earliest detectable increase occurs by 1¼ hours and is maintained for at least 24 hours. This increase chronologically parallels the induction of tyrosine aminotransferase in the cells. In order to clarify the mechanism of the increase in phenylalanine acceptor activity, highly purified transfer RNAs and aminoacyl-synthetases were isolated from the cytosol of steroid treated and control HTC cells. A reproducible 20-40% increase in phe-tRNA was demonstrated for steroid treated cells using an in vitro charging system in which tRNA was shown to be limiting. There were no changes in aminoacyl-synthetases in dexamethasone treated cells, nor was an increase in other tRNAs found. In vivo induction of the increase in phe-tRNA was blocked by actinomycin-D. Isoaccepting forms of phe-tRNA are currently being fractionated on reversed phase chromatographic columns. Glucocorticoids may exert a macroregulatory effect on the synthesis of diverse proteins by altering the transcription, processing, methylation or degradation of a tRNA which is rate limiting for the translation of specific mRNAs.

13. Interaction of glucocorticoids with rat liver nuclei in vitro

M. BEATO and P. FEIGELSON, *Institute of Cancer Research and the Department of Biochemistry, Columbia University, New York, N.Y., U.S.A.*

The uptake in vitro of glucocorticoids by purified rat liver nuclei is dependent on the concentration of steroid. Two classes of binding sites can be detected; one, present in limited number, with high affinity ($\simeq 10^{-8}$ M) and specificity for active glucocorticoids and the other nonsaturated at 10^{-4} M cortisol, with low steroid specificity. Using a procedure which allows selective estimation of the high affinity binding sites, it can be shown that their number per cell nucleus markedly decreases after adrenalectomy. The binding capacity of nuclei from adrenalectomized rats can be restored in several days to the values of intact animals by daily injections of cortisol. Addition to the in vitro incubation medium of the macromolecular fraction of liver cytosol enhances glucocorticoid binding to purified nuclei from adrenalectomized rats to a greater extent than to nuclei from intact animals. A macromolecular component(s) of liver cytosol, different from transcortin, appears to be involved in this enhanced nuclear binding of steroids.

14. Hormonal control of adrenal RNA polymerase activity

S. A. FUHRMAN and G. N. GILL, *School of Medicine, University of California at San Diego, La Jolla, Calif., U.S.A.*

To investigate the regulation of RNA synthesis by ACTH and cAMP, the adrenal RNA polymerase enzymes were characterized and the effect of hormone administration on polymerase activity studied. Two peaks of RNA polymerase were identified by DEAE-Sephadex chromatography of material purified from bovine adrenal cortical nuclei. The first peak is resistant to α-amanitin and corresponds to nuclear ribosomal RNA polymerase I; the second peak is sensitive to α-amanitin and corresponds to nucleoplasmic RNA polymerase II. The effects of ACTH and dibutyryl cAMP administration to rats and guinea pigs were investigated in purified adrenal nuclei using α-amanitin to discriminate between the two RNA polymerase activities. ACTH administration results in a three fold increase in the activity of RNA polymerase I at 14 hrs; no change in RNA polymerase II activity occurs.

Dibutyryl cAMP causes a similar stimulation of RNA polymerase I activity in hypophysectomized rats. The ACTH induced increase in RNA polymerase I activity is blocked by cycloheximide, but is unaffected by aminoglutethimide. These studies, which characterize two adrenal RNA polymerase enzymes, demonstrate preferential regulation of ribosomal RNA polymerase activity by ACTH and cAMP. The hormonal control of RNA synthesis requires protein synthesis, but is independent of steroidogenesis.

15. Hormonal modification of mouse liver lysosomal protein metabolism by cortisone acetate

B. S. CHERTOW, W. E. BUCHANAN and T. B. SCHWARTZ, *Madigan General Hospital, Tacoma, Wash. and Rush-Presbyterian-St. Luke's Medical Center, Chicago, Ill., U.S.A.*

The effect of cortisone acetate (CA) on hepatic lysosomal protein metabolism and its relation to stabilization of the lysosomal membrane were studied in mice. Denatured iodinated-125 human serum albumin (IHSA) was injected by tail vein and large granule fractions containing lysosomes were isolated and incubated at pH 7 and 37°C for 1 hour. The IHSA uptake and degradation were measured as total and TCA-soluble released counts, respectively. Mice pretreated with CA, 20 mg i.p. daily in divided doses, showed a 32% increase in degradation in vitro. Mice pretreated with CA, 5 mg i.p. daily for 4 days, showed a 23% increase in degradation in vitro, 29% increase in degradation in vivo, and 73% increase in IHSA uptake. Although vitamin A, a lysosomal labilizing agent, decreased IHSA degradation, CA did not antagonize the effect of vitamin A. Hydrocortisone, 3.6×10^{-3} M in vitro, did not increase IHSA breakdown. CA did not decrease the nonsedimentable catheptic activity determined immediately after sacrifice. Thus, a catabolic effect of CA on mouse liver protein metabolism is mediated through the lysosome but, contrary to a generally held view, is not associated with membrane stabilization, although the integrity of the lysosomal membrane is necessary for optimal lysosomal catheptic activity.

16. On the mechanism of action of cortisol on glucose metabolism by adipose tissue

A. KAWAI, *The Institute for Adult Diseases, Asahi Life Foundation, Tokyo, Japan*

In order to explore the mechanism by which glucocorticoid exerts an inhibitory action on glucose metabolism by adipose tissues, the effects of (1) adipose cell size, (2) proteolytic treatment of the tissue and (3) linkage of cortisol to beaded agarose prepared by the method of Rosner and Bradlow on the response of glucose utilization by rat epididymal adipose tissue to the hormone were studied. Judging from the slopes of decreasing curves of ^{14}C-glucose conversion to total lipids in response to increasing amount of cortisol, its responsiveness appeared to be related to adipose cell size, the smaller the adipose cells the more cortisol sensitive was the tissue. Treatment of the adipose tissue fragments with trypsin (1 mg/50 mg tissue) for 5 min at 37 °C did not hinder the inhibitory action of cortisol. After prolonged incubation (120 min), however, cortisol was without significant effect. Cortisol linked to amino Sepharose was shown to depress substantially the metabolism of glucose by the adipose tissue. These results indicate that although the cell membrane may be an important site of the inhibitory action of the glucocorticoid, since this was dependent upon the cell size, entrance of cortisol into the cell is not a prerequisite for the action.

17. Cortisol effects upon liver and muscle. Modulation by the nutritional state

J. HANOUNE and A. M. CHAMBAUT, *Institut de Pathologie Moléculaire, Paris, France*

The metabolic responses to cortisol administration are well known in normal starved rats. We assessed the possible role of complex physiological interactions in these responses by studying the effect of cortisol in glucose-loaded rats. When administered together with cortisone, glucose strongly altered tyrosine transaminase induction as well as most of the early hepatic parameters of the glucocorticoid action. Similarly, glucose inhibited the catabolic effect of cortisol upon radioactive leucine incorporation into muscle protein without modifying the hormonal effect upon AIB accumulation. The effect of glucose did not appear

to be due to an impaired radioactive cortisol metabolism in liver or muscle. The role of the pancreatic hormones in the glucose effect was assessed; after the glucose load, the plasma levels of insulin and enteroglucagon rose while the level of pancreatic glucagon was depressed. The effect of glucose on the hepatic responses to cortisol administration was largely prevented by simultaneous injection of glucagon. It is suggested that the nutritional state of the organism can modulate the biochemical responses to cortisol administration. A complex model of plurihormonal interaction, both at the transcriptional and at the translational level, upon a single enzyme such as tyrosine transaminase is proposed.

18. Effects of cortisol on glycogen synthesis and gluconeogenesis in fetal rat liver

C. MONDER and A. COUFALIK, *Hospital for Joint Diseases, Mt. Sinai School of Medicine, New York, N.Y., U.S.A.*

Cortisol stimulated glycogen synthesis in 21 ± 1 day old fetal rat liver in organ culture. The increase was proportional to the amount of glucose present in the medium, occurred slowly over 44 hr, and was accelerated after prior incubation of explants with no steroid. Glucose-U-^{14}C in the medium and glycogen formed from it had the same specific activity whether or not cortisol was present. Fetal livers synthesized glucose and glycogen from L-alanine-U-^{14}C. Cortisol did not affect gluconeogenesis, but increased the proportion of label incorporated into glycogen, and decreased incorporation into glucose. Accumulation of glycogen under all conditions studied was proportional to glycogen synthetase b activity. It is concluded that (*a*) the major source of glycogen in fetal rat liver is glucose; (*b*) synthesis of glycogen from glucose is stimulated by cortisol; (*c*) gluconeogenesis takes place to a measurable extent and is not influenced by cortisol; (*d*) cortisol directs a common intermediate towards glycogen synthesis and away from glucose formation. (Supported by USPHS Grant AM09006.)

19. Two receptors in the mechanism of corticosterone action in rat liver

R. S. SNART and R. E. SHEPHERD, *Department of Zoology, University of Sheffield, Sheffield, U.K.*

We have characterized the two physiologically active receptor proteins identified in our earlier studies of corticosterone binding in rat liver. The major receptor has been shown by various chromatographic procedures to be a basic protein (mol.wt. 100,000); it has no subunit characteristics and is cytonuclear but shows no specific nuclear uptake when activated by the hormone. The binding of corticosterone to this receptor protein has been characterised by a K_{ass} value of 5×10^8 l/mole. The second receptor is present at very low concentrations and was not seen in our chromatographic separations. However, an acidic protein fraction may be obtained from chromatin preparations that specifically binds corticosteroid and would correspond to our more limited high affinity binding sites K_{ass} 10^{10} l/mole. It has not been possible to study any cytonuclear characteristics of this acidic protein. It may, however, be compared with acidic receptors characterised in other target tissues and considered to act as a derepressor in the hormonal control of protein synthesis. The presence of a basic receptor suggests that such protein-hormone complexes may act as repressor molecules to control the synthesis of post transcriptional inhibitors such as that involved in the translational control of tyrosine transaminase. The role of two receptors in the mechanism of hormonal action may be to allow control of protein synthesis at the transcriptional and translational level.

20. Keratinization-inducing effect of hydrocortisone on chick embryonic skin growing in a chemically defined medium

H. ENDO, M. SUGIMOTO and K. TAJIMA, *Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences and Department of Dermatology, Faculty of Medicine, University of Tokyo, Tokyo, Japan*

13-day chick embryo shank skin was cultured for 4 days in a chemically defined medium. Our earlier studies demonstrated that hydrocortisone of minimal concentrations in the medium induced dramatic histological keratinization of the epidermis. In this work the effect

of hydrocortisone was biochemically and electronmicroscopically examined. The amino acid composition of the epidermal proteins indicated that the content of glycine was markedly increased by the steroid. The chemical analyses, together with studies of ^{3}H-glycine incorporation into the protein, strongly suggested that hydrocortisone might enhance the synthesis of some glycine-rich protein(s). When S-carboxymethyl epidermal protein of the cultured skin was examined by polyacrylamide gel disc electrophoresis, the steroid was clearly shown selectively to increase a limited part of the specific protein which is rich in glycine (about 25%) and of fibrillar nature. Electronmicroscopical examination of the cultured skin revealed that the steroid treatment resulted in the formation of a great number of tonofibrils within the basal cells of the epidermis.

21. Differential hormonal control of histidase in liver and epidermis during postnatal development

M. FEIGELSON, *Departments of Biochemistry and Obstetrics-Gynecology, Harlem Hospital, and the International Institute for the Study of Human Reproduction, Columbia University, New York, N.Y., U.S.A.*

In the course of investigation of differentiation of the mechanisms which regulate enzymes, the postnatal development of histidase and its responses to various hormonal influences were compared in rat liver and epidermis. Whereas histidase activities in liver markedly increase during maturation of weanlings to adults, enzyme activities in epidermis diminish during the same period. In both tissues, histidase activities of adult females are double those of males and enzyme levels rise following hypophysectomy, suggesting similar gonadal and hypophyseal regulation of this enzyme in these two target tissues. However, cortisol and glucagon enhance histidase activities in livers of young rats, but have little effect on enzyme activities in the epidermis of the same animals. Cortisol elevates liver histidase only in immature rats; its inducing ability wanes at adulthood. This age-dependent sensitivity to glucocorticoid may play a significant role in the developmental rise and subsequent plateau in activity of the hepatic enzyme. Conversely, the observed refractoriness of the skin enzyme to glucocorticoid and/or glucagon may account for its failure to rise during this period. These findings suggest that differentiation of hormonal regulation during maturation may participate in determining patterns of enzymic development in various tissues.

Session C 3

Steroid Biosynthesis and Secretion

(Abstracts 22-31)

22. Localization of steroid biosynthetic enzymes in membranes of testicular endoplasmic reticulum

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Microsomal fractions were prepared from mouse testes stimulated by HCG to produce hypertrophied interstitial cells filled with smooth endoplasmic reticulum (SER). These were incubated with either pregnenolone-^{3}H(preg)+progesterone-^{14}C (prog) or progesterone-^{14}C+17-hydroxyprogesterone-^{3}H (17OHprog) under conditions of minimal metabolism ($< 5\%$) and of metabolism to androstenedione (andr) and testosterone (testos). Isotope ratios and concentrations in both supernatant and pellet were determined. When no metabolism occurred the order of pellet:supernatant ratios was preg > prog > 17OHprog, the latter apparently simply diffusing. When a mixture of preg and prog was metabolized the isotope ratio in prog, 17OHprog, andr and testos resembled that of the 2 substrates in the super rather than in the pellet. When a mixture of prog and 17OHprog was metabolized the isotope ratios in andr and testos resembled that of the substrates in the pellet rather than in the super. The data suggest a localization of the 3β-hydroxysteroid dehydrogenase system on the outer surface of the vesicles while the 17β-hydroxylase appears to be on the inner surface. Preg and prog are concentrated in the vesicle while the other metabolites appear to diffuse freely. A partial picture of intracellular circulation of steroids in the interstitial cell during androgen biosynthesis has been derived.

23. Isotope effects in steroid hydroxylation

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We are concerned with the effects of hydrogen-isotope labeling on steroid metabolism. Incubation of estr-4-ene-3,17-dione-7β-^{3}H-4-^{14}C with *B. malorum* resulted in a 1.3-fold increase in the ^{3}H/^{14}C ratio in 6β-hydroxyestrenedione. We report now that another product, estr-4-ene-3,11,17-trione, also had a marked increase in its ratio. Incubation of 6β,7β-ditritiated- or dideuterated-estrenedione gave the 7β-hydroxy product with loss of over 50% of the label at C-6. The 11-keto product showed a marked increase in hydrogen-isotope label as before. Incubation of 6β-deuteroestrenedione gave the 7-hydroxy material without loss of label at C-6 thus linking the decrease in label at this position with hydrogen-isotope labelling at C-7β. This in turn suggested the operation of a primary isotope effect which by retarding hydroxylation at C-7, decreased the participation of labeled materials in this reaction. GLC analysis of the products confirmed that hydroxylation decreased substantially at the positions labeled with deuterium. The recovered substrate in all cases did not show an increase in concentration of deuterium or tritium. This, together with the increases in label noted, suggest that 7β-, 6β- and 11-hydroxylation are carried out by one enzyme or a closely related enzyme complex which is capable of shifting reactions away from those involving labeled positions.

24. Evidence for a squalene and cholesterol independent pathway of steroid biosynthesis in the pregnant mare

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Our previous studies indicated that the ring B unsaturated estrogens, equilin (Eq) and