GREENBERG 1974[11], there was a good correlation between the phagocytic index and morphological evidence of erythrophagocytosis in the presence or absence of cytochalasin B. In control experiments, cytochalasin B was not found to cause any metabolic effect, at the concentration used, except for inhibition of phagocytosis. However, the phagocytosis induced by a series of different anti-D sera tested was readily detected, the phagocytic indices varied from 1.23 to 1.52. Heat inactivation of anti-D sera (56 °C for 30 min) led to less phagocytosis (Figure a). As various authors (HOLM[8], HUBER and FUDENBERG[9]) have shown that the presence of free IgG in the incubation mixture can block effector cells, a further series of experiments were performed in the absence of serum, pretreating the erythrocytes (CDe/cDE) with anti-D serum for 30 min, washing and then adding the phagocytic cells. Phagocytosis of these presensitized red cells by buffy coat leukocytes was increased compared to experiments where serum was present for the whole incubation period (phagocytic indices varying from 1.37 to 1.60). Nevertheless, if either anti-D or normal serum was added to the culture of buffy coat cells and presensitized red cells for the whole period of experiments, phagocytosis was inhibited and this reduction was even greater than that when anti-D serum was heat inactivated (Figure b).

In the series of experiments where monocytes were used as phagocytic cells in culture with presensitized red blood cells, phagocytosis was greater than that seen with buffy coat cells (ratio 1.55 to 1.90) (Figure c). It was also observed that purified monocytes in the presence of anti-D serum during the whole incubation period were still able to phagocytose Rh-positive red cells (phagocytic ratio 1.58; not shown). As in the previous experiments with buffy coat leucocytes, the presence of anti-D or AB serum in the incubation mixtures of monocytes and presensitized red cells reduced phagocytosis. An explanation for this could be that when the binding sites of the target cells are saturated, the addition of free IgG antibodies or complexes would block the Fc receptors of the effector cells. Some other authors[12] have also reported that this inhibitory effect of IgG seems to depend on the degree of red cell sensitization. Moreover, this phagocytic activity was partly dependent on the presence of complement, since heat inactivated sera were less effective. An alternative explanation for this could be that aggregated IgG blocks the Fc receptors of the monocytes. However, in the series of experiments performed in order to show extracellular lysis of anti-D sensitized red blood cells, poor chromium was released from target cells as compared with control pooled AB serum. Nevertheless, in accordance with HOLM[8], more significant haemolytic activity was detected by pretreatment of red cells with anti-D serum and culturing in the absence of serum thus avoiding its inhibitory effects (unpublished observations).

However, the mechanism by which anti-D coated red cells are removed from the circulation still remains unclear. We believe that both mechanisms of haemolysis extracellular and intracellular are involved in the process, and our data support the concept that with IgG antibody coated red cells, one of the main mechanism of removal is by phagocytosis.

Summary. In order to determine the phagocytosis of human anti-D coated red cells we adopted a quantitative technique for its measurement using human erythrocytes labelled with ^{51}Cr-chromate to support the assumption that erythrophagocytosis is one of the main mechanisms by which IgG-anti-D-antibody coated red cells are destroyed.

E. FERNANDEZ-CRUZ[13,14], A. H. GREENBERG and J. BROSTOFF

Department of Immunology, Middelesex Hospital Medical School, London W1 (England), and Department of Immunology, Clinica Puerta de Hierro, Autonomous University, San Martin de Porres 4, Madrid 35 (Spain), 6 May 1975.

[11] A. H. GREENBERG, in press (1975).

[12] H. HUBER, Science *162*, 1281 (1968).

[13] Acknowledgments. I wish to express my thanks to Prof. I. ROITT and to Prof. J. M. SEGOVIA DE ARANA for their valuable suggestions and for their encouragement. Dr. E. FERNANDEZ-CRUZ is supported by the March Foundation of Spain.

[14] Address for reprint requests. Dr. E. Fernandez-Cruz, Department of Immunology, Clinica Puerta de Hierro, San Martin de Porres, 4, Madrid 35, Spain.

The Effect of Luteineizing Hormone on Chicken Testis and Pineal Gland Uptake of ^{32}P[1]

An increasing number of investigations of the pineal gland have been reported since the discovery of a potential pineal hormone (melatonin)[2] and the demonstration of a relationship between ambient lighting and the biosynthetic activity of the pineal parenchyma[3]. The existence of one or more gonad inhibiting polypeptides in the mammalian pineal gland has been established[3–7]. Various pineal indoleamines such as melatonin have also been shown to possess anti-gonadotropic qualities[3,8,9]. This effect may vary with age. For example, RATHKAMP[10] has shown that the pineal of the White Leghorn cockerel stimulates gonadal activity between 9 and 17 days post-hatching and inhibits gonadal activity after 17 days post-hatching. It has also been observed that the pineal organ may be a target organ of testicular hormones[11–14].

The glandular uptake of ^{32}P, administered carrier-free, has been used in our laboratory as an indicator of increased or decreased organ metabolism[15,16]. This method was used in these studies to determine the effect of luteinizing hormone on the pineal gland and testis of 3-, 10- and 13-day-old White Leghorn cockerels (*Gallus domesticus*).

Materials and methods. White Leghorn cockerels were purchased from the Indiana Farm Bureau Co-op, Indianapolis, Indiana, within 12 h of hatching. All birds used in these studies were housed in brooders under controlled temperature (21–24 °C) and lighting (14 h light: 10 h dark). Food and water were available ad libitum until 12 h before autopsy at which time all birds were taken off feed.

Eighty 13-day-old cockerels were divided into 5 groups of 16 birds each. An injection of 30 μg LH (NIH-LH-S18)[17] was administered to each bird of a given group at either 1.0, 2.0, 4.0 or 8.0 h before autopsy. As a control the 5th group did not receive LH treatment (Table I).

In a 2nd experiment, sixty-four 13-day-old cockerels were divided into 4 groups of 16 birds each. An injection of 5, 10 or 20 μg LH (NIH-LH-S18) was administered to

each bird in a given group 4.0 h before autopsy. The 4th group did not receive LH treatment and served as a control (Table II).

In the 3rd experiment, thirty-two 3-day-old cockerels and thirty-two 10-day-old cockerels were divided into groups of 16 birds each. An injection of 20 µg LH (NIH-LH-S18) was administered 4.0 h before autopsy to each of the 16 birds at each age. As controls, the remaining birds at each age did not receive LH treatment (Table III).

All injections were administered s.c. in a volume of 0.2 ml distilled water. Each bird received a single s.c. injection of 1.0 µCi ^{32}P (New England Nuclear Chemicals) in 0.2 ml distilled water. The birds were killed by cervical dislocation 1.0 h following the ^{32}P injection. Body weights were recorded and pineal glands and testes were quickly excised, cleaned and weighed to the nearest 0.01 mg on a Roller Smith torsion balance and placed on a planchet. After air-drying, the radioactivity of each organ was determined on a Chicago-Nuclear automatic gasflow counter. Results were recorded in cpm minus background divided by the wet organ weight in milligrams (cpm-BG)/mg. Means and standard errors (SE) were calculated [$SE = \sqrt{\Sigma d^2/N(N-1)}$] and significance of the data was determined by employing Student's two-tailed *t*-test[18].

Table I. Responses of 13-day-old cockerel testis and pineal glands to luteinizing hormone administered at various times before autopsy[a]

Group ($N = 16$)	Body weight (g)	Pineal response (cpm-BG)/mg	Testis response (cpm-BG)/mg
Control	64 ± 1[b]	22.21 ± 0.90	6.21 ± 0.52
1.0 h	61 ± 1	19.18 ± 1.02	6.93 ± 0.93
2.0 h	62 ± 2	20.26 ± 1.05	6.88 ± 0.98
4.0 h	60 ± 2	14.78 ± 1.10[d]	8.75 ± 1.03[e]
8.0 h	63 ± 1	22.14 ± 1.08	7.01 ± 1.01

[a] A1.0 h uptake of 1.0 µCi ^{32}P was used. 30 µg LH was administered at the various times before autopsy. [b] Data are expressed as means ± SE. [d] Significantly different from the control ($p > 0.05$). [e] Significantly different from the control ($p > 0.005$).

Table II. Responses of 13-day-old cockerel testis and pineal glands to different doses of luteinizing hormone[a]

Group ($N = 16$)	Body weight (g)	Pineal response (cpm-BG)/mg	Testis response (cpm-BG)/mg
Control	61 ± 2[b]	15.02 ± 0.08	5.30 ± 0.61
5 µg LH	60 ± 2	14.92 ± 0.47	5.72 ± 0.95
10 µg LH	63 ± 2	13.61 ± 0.92	6.35 ± 1.02
20 µg LH	59 ± 1	7.09 ± 1.17[c]	7.82 ± 0.91[c]

[a] A 1.0 h uptake of 1.0 µCi ^{32}P was used. LH was administered 4.0 h before autopsy (Table I). [b] Data are expressed as means ± SE. [c] Significantly different from the control ($p > 0.001$).

Table III. Responses of 3- and 10-day-old cockerel testis and pineal glands to luteinizing hormone[a]

Group ($N = 16$)	Body weight (g)	Pineal response (cpm-BG)/mg	Testis response (cpm-BG)/mg
3 day Control	37 ± 2[b]	12.63 ± 1.31	6.60 ± 0.38
3 day LH-treated	33 ± 2	14.74 ± 1.05	13.07 ± 0.60[c]
10 day Control	66 ± 2	5.86 ± 0.07	2.54 ± 0.27
10 day LH-treated	64 ± 2	6.81 ± 1.07	7.09 ± 1.99[d]

[a] A 1.0 h uptake of 1.0 µCi ^{32}P was used. 20 µg LH was administered at 4.0 h before autopsy. [b] Data are expressed as means ± SE. [c] Significantly different from the control ($p > 0.001$). [d] Significantly different from the control ($p > 0.05$).

Results and discussion. Luteinizing hormone decreased pineal uptake of ^{32}P and increased testis uptake of ^{32}P in the 13-day-old cockerel. This effect was maximum when 20 µg LH was administered 4.0 h before autopsy (Table I and II). Pineal uptake of ^{32}P was not significantly altered by treatment with LH in the 3- and 10-day-old groups although testis uptake of ^{32}P increased significantly in both age groups (Table III).

LH treatment stimulates the testes to produce increased amounts of androgens. These are believed to feedback on the hypothalamus, decreasing gonadotropin releasing hormone (Gn-RH) levels. This in turn results in a decrease in the production of LH by the anterior pituitary. Recent studies have indicated that the pineal organ is also a target organ of gonadal hormones. Protein receptors for sex steroids have been observed in the mammalian pineal gland and found to be similar to those present in the uterus, prostate and hypothalamus[11–13]. It has also been shown that pinealectomy increases plasma testosterone levels in male rats[19]. Furthermore, castration of pre-pubertal rats resulted in hypertrophy of pinealocytes[14].

WHITE et al.[20] observed gonadotropic releasing hormones in the pineal organ of at least 3 mammals in quantities 4–10 times that of the corresponding hypothalami. Others have reported similar pineal Gn-RH stores, although to a lesser extent[21]. Recent reports indicate a negative feedback mechanism of testicular androgens on the pineal organ[22].

[1] Contribution No. 1004 from the Department of Zoology, Indiana University, Bloomington, Indiana 47401, USA.
[2] A. B. LERNER, J. D. CASE, Y. TAKAHASHI, T. LEE and W. MORI, J. Am. chem. Soc. *80*, 2587 (1958).
[3] R. J. WURTMAN, J. AXELROD and L. S. PHILLIPS, Science *142*, 1071 (1963).
[4] I. EBELS, Gen. comp. Endocr. *25*, 189 (1975).
[5] B. BENSON, M. J. MATTHEWS and A. E. RODIN, Acta endocr. Copenh. *69*, 257 (1972).
[6] R. J. ORTS, B. BENSON and B. F. COOK, Life Sci. *14*, 1501 (1974).
[7] D. W. CHEESMAN and P. H. FORSHAM, Proc. Soc. exp. Biol. Med. *147*, 438 (1974).
[8] A. MOSZKOWSKA and I. EBELS, J. neurovisc. Relat. Suppl. *10*, 160 (1971).
[9] R. J. REITER, M. K. VAUGHAN, D. E. BLASK and L. Y. JOHNSON, Science *185*, 1169 (1974).
[10] W. R. RATHKAMP, Doctoral Thesis, Indiana University (1972).
[11] G. RASPE, *Advances in the Biosciences* (Pergamon Press, London and Vieweg, Braunschweig 1971), vol. 7.
[12] P. J. THOMAS, J. Endocr. *57*, 333 (1973).
[13] D. P. CARDINALI, C. A. NAGLE and J. M. ROSNER, Life Sci. *16*, 93 (1975).
[14] J. DAS GUPTA, J. Endocr. *41*, 607 (1968).
[15] F. J. ZELLER, Proc. Indiana Acad. Sci. *72*, 257 (1963).
[16] W. R. BRENEMAN and W. R. RATHKAMP, Biochem. biophys. Res. Commun. *52*, 189 (1973).
[17] Appreciation is expressed to the Pituitary Distribution Program of the National Institute of Arthritis and Metabolic Disease for the LH used in this study.

Data presented in this paper indicates that the pineal gland of the 13-day-old cockerel is affected, either directly or indirectly, by the administration of luteinizing hormone. Since it has been shown that LH has no effect on pineal uptake of ^{32}P in vitro[10] it may be hypothesized that the exogenous LH causes an increase in release of testicular hormones which then decrease pineal activity via a negative feedback mechanism. Furthermore, this decreased pineal activity may be a decrease in release or synthesis of Gn-RH which would decrease pituitary LH production.

The results obtained from 3- and 10-day-old birds indicate that this mechanism may not be developed until about 13 days post-hatching. Differences in pineal and testis uptake of ^{32}P by 3- and 10-day-old birds are probably due to the differences in body weights (Table III).

Future studies will attempt to further define the pineal-gonadal relationship.

Summary. The glandular uptake of radioactive phosphorus (^{32}P), administered carrier-free, was used as an endpoint for the study of the effects of luteinizing hormone (LH) on the testis and pineal gland of 3-, 10- and 13-day-old White Leghorn cockerels. Pineal uptake of ^{32}P of the 13-day-old birds decreased and testis uptake of ^{32}P increased following LH treatment. Maximum effects were observed when 20 µg LH was administered 4.0 h before autopsy. Although testis uptake of ^{32}P increased following LH treatment in 3- and 10-day-old cockerels, pineal uptake of ^{32}P remained unchanged.

J. W. SACKMAN

Indiana University, Department of Zoology, Jordan Hall 224, Bloomington (Indiana 47401, USA), 17 July 1975.

[18] G. W. SNEDECOR, *Statistical Methods*, 5th edn. (Iowa State College Press, Ames, Iowa 1956).
[19] C. A. NAGLE, D. P. CARDINALI, N. P. LABORDE and J. M. ROSNER, Endocrinology *94*, 294 (1974).
[20] W. F. WHITE, M. T. HEDLUND, G. F. WEBER, R. H. RIPPEL, E. S. JOHNSON and J. F. WILBER, Endocrinology *94*, 1422 (1974).
[21] N. P. PLOTNIKOFF, A. J. PRANGE, JR., G. R. BREESE, M. S. ANDERSON and I. C. WILSON, Science *178*, 417 (1972).
[22] D. P. CARDINALI, C. A. NAGLE and J. M. ROSNER, Gen. comp. Endocr. *26*, 50 (1975).

Corticosteroid-Like Effect of Cyproterone and Cyproterone Acetate in Mice

Cyproterone (6 chloro δ^{6}-1,2 α-methylene, 17 α-hydroxy progesterone SH 80 881 Schering) and cyproterone acetate (17 α-acetoxy, 6 chloro, 1α, 2α-methylene 4,6 pregnandiene 3,20 dione SH 80 174) are two of the most active antiandrogenic steroids blocking the peripheral action of both endogenous and exogenous testosterone[1–3]. Their blocking effect is manifested in the peripheral target tissues, the seminal vesicles, the prostate and sebaceous glands. The majority of experiments suggest that the mechanism of the antiandrogenic action of cyproterone acetate consists in competitive inhibition of the action of the androgen testosterone or dihydrotestosterone at androgen receptor sites in the target organs. Cyproterone acetate is not only an antiandrogen but also a powerful gestagen. Free alcohol cyproterone is a weaker antiandrogen but has no gestagen activity[1]. Cyproterone acetate was reported to decrease the adrenal weight and level of circulating corticosteroids in the rat[1, 2, 5, 6].

In order to ascertain the corticosteroid-like activity of cyproterone acetate and cyproterone, the drug was administered to intact male mice of strain H (Velaz, Prague) weighing 40 g. The animals were fed a standard laboratory diet (Velaz) containing 23% protein with water ad libitum, and were kept in an indirectly illuminated room with a controlled temperature of 24 + 2 °C. The mice were divided into 4 groups of 8 each. Cyproterone acetate Schering SH 80 714 and cyproterone Schering SH 80 881 were administered in a standard laboratory diet in doses of 5 mg/day p. animal for 21 days. Corticoid prednisone Spofa was given in the food in a dose of 1 mg/day p. mouse for 21 days. The animals were weighed before and after the experiment and their food consumption was checked daily. No food was left, so

[1] W. ELGER, R. VON BERSEWORDT-WALLRABE and F. NEUMANN, Naturwissenschaften *54*, 549 (1967).
[2] F. NEUMANN and W. ELGER in *Androgens in Normal and Pathological Conditions* (Ed. A. VERMEULEN, Excerpta Med. Int. Congr. Ser. Amsterdam 1965), No. 101, p. 168.
[3] F. NEUMANN, W. ELGER and H. STEINEBOCK, Phil. Trans. Soc. London B *259*, 179 (1970).
[4] C. DENEF, M. VANDEPUTTE and P. DE MOOR, Endocrinology *83*, 945 (1968).
[5] J. LERNER, Recent Progr. Horm. Res. *20*, 435 (1964).
[6] L. STÁRKA, K. MOTLÍK and V. SCHREIBER, Physiologia bohemoslov. *21*, 233 (1972).

Group	Body weight final (g)	Seminal vesicle (mg/100 g)	Adrenal (mg/100 g)	Spleen (mg)	Spleen (mg/100 g)	Dry spleen (mg/100 g)
Controls	37.5 ± 2.88 (4)	474.9 ± 72.4 (2–4)	15.6 ± 1.8 (2, 4)	211.4 ± 52.4 (2–4)	562.8 ± 145.9 (2–4)	142.0 ± 36.0 (2–4)
Cyproterone acetate	34.0 ± 2.68 (3)	210.6 ± 37.5 (1, 3, 4)	8.6 ± 1.5 (1, 3)	53.2 ± 7.8 (1, 3)	157.7 ± 26.7 (1, 3)	38.8 ± 6.3 (1, 3)
Cyproterone	38.1 ± 2.10 (2, 4)	285.8 ± 32.8 (1, 2, 4)	13.7 ± 3.6 (2, 4)	125.0 ± 25.3 (1, 2, 4)	327.2 ± 60.5 (1, 2, 4)	82.8 ± 15.4 (1, 2, 4)
Prednisone	33.3 ± 1.4 (1, 3)	630.2 ± 120.9 (1–3)	7.5 ± 0.8 (1, 3)	52.8 ± 5.3 (1, 3)	158.7 ± 18.2 (1, 3	39.7 ± 4.1 (1, 3)

Means ± 95% confidence limits. The numbers of groups with statistically different means are given in brackets.