

sommaton d'O<sub>2</sub> pendant la même période est multipliée par 19; la quantité d'azote tissulaire, qui représente la masse de protoplasme actif est multipliée, dans le même temps, par 18 environ. La glycolyse anaérobie ne suit pas, comme le fait la respiration oxydative, la croissance biochimique tissulaire.

Du stade 28 au stade 36, la quantité d'acide lactique produite rapportée à 1 µg d'azote total diminue rapidement, puis à partir de ce dernier stade jusqu'au deuxième jour après l'éclosion cette quantité reste pratiquement constante. Cependant, si l'on considère les quantités d'acide lactique libérées rapportées à 1 µg de phosphore de l'acide désoxyribonucléique, ce qui est l'expression de l'activité glycolytique au niveau des cellules du mésencéphale embryonnaire, on constate que du stade 28 au stade 36 cette quantité est pratiquement constante et égale à 12,2 µg en moyenne, puis, de ce dernier stade à l'éclosion elle s'accroît du double. Or, pendant cette dernière période de développement embryonnaire l'un de nous a déjà montré que la consommation d'oxygène des lobes optiques est multipliée par 3 environ<sup>1</sup>.

**Conclusions.** Les résultats que nous venons d'exposer sont par conséquent en étroit accord avec ceux obtenus *in vitro* par d'autres auteurs sur le cerveau du fœtus de chat, de lapin et de chien<sup>8</sup> et sur le cortex cérébral du fœtus de cobaye<sup>9</sup>. Au niveau des lobes optiques de l'embryon de poulet, la période de multiplication des neuroblastes allant sensiblement jusqu'au dixième jour d'incubation (stade morphologique 35–36)<sup>1,4</sup> est caractérisée, du point de vue du métabolisme énergétique, par une supériorité de la glycolyse anaérobie (schéma d'Emden-Meyerhof). Du dixième jour d'incubation jusqu'à l'éclosion et même au-delà, période qui correspond à la phase de

différenciation des neuroblastes en neurones<sup>1,4</sup> à activité fonctionnelle<sup>2,10</sup>, prédominent les mécanismes de la phosphorylation oxydative (cycle de l'acide citrique) fournissant la quantité suffisante de molécules d'adénosine triphosphate (ATP) indispensables à la croissance des expansions nerveuses<sup>1,4,11</sup>, ainsi qu'à l'établissement de l'activité fonctionnelle<sup>2,10,12</sup>.

**Summary.** A series of *in vitro* experiments have been made on lactic acid production in anaerobiosis in the developing optic lobes (mesencephalon) of the chick embryo. The rate of anaerobic glycolysis is relatively important during multiplication of the neuroblasts; but, during the differentiation of the neuroblasts into mature neurons, the anaerobic processes are lower than the phosphorylation and oxidation mechanisms essential for the growth of nerve expansions and onset of functional activity.

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<sup>8</sup> H. E. HIMWICH, *Brain Metabolism and Cerebral Disorders* (Williams and Wilkins, Baltimore 1951).

<sup>9</sup> L. B. FLEXNER, J. B. FLEXNER et L. HELLERMAN, *J. cell. comp. Physiol.* 47, 469 (1956).

<sup>10</sup> A. BONICHON, *J. Neurochem.* 5, 195 (1960).

<sup>11</sup> V. B. PETERS et L. B. FLEXNER, *Amer. J. Anat.* 86, 133 (1950).

<sup>12</sup> L. B. FLEXNER, D. B. TYLER et L. J. GALLANT, *J. Neurophysiol.* 13, 427 (1950).

### The Antigenicity of Sheep Follicle-Stimulating Hormone

The antigenic composition of purified sheep follicle stimulating hormone (FSH) and luteinizing hormone (LH) has been investigated by SEGAL et al.<sup>1</sup>. They observed that ovine FSH had a common antigen with ovine LH. These investigators have further shown that the FSH had an LH activity that could be selectively absorbed by the antiserum to LH. SEGAL et al.<sup>2</sup>, in their more recent studies, have used an antiserum to FSH absorbed with blood serum. RAO and SHAHANI<sup>3</sup> observed that human chorionic gonadotrophin (HCG) had a minimum of 3 antigens in common with blood serum and these could be removed by absorbing the antiserum to HCG with normal blood serum. The studies reported here were carried out to find whether ovine, bovine, porcine and human pituitary FSH and LH have common antigens, and whether the serum contaminants in the hormone preparations could be selectively removed by absorbing the specific antisera with sheep serum.

Antisera to the hormone and sheep serum were obtained from rabbits immunized with the respective gonadotrophins and the serum along with Freund's complete adjuvant. The characterization of antigens was carried out both by the Ouchterlony gel diffusion technique<sup>4</sup> and the immuno-electrophoretic technique<sup>5</sup>.

Experiments were carried out to study the common antigens ovine FSH has with ovine LH, bovine LH, porcine LH, ram and sheep serum. The antiserum to FSH was placed in the centre of an agar-plate (Figure 1), and

around it were placed ovine FSH, ovine LH, bovine LH, ram serum, porcine LH and sheep serum. The antiserum gave five precipitin lines with ovine FSH and one precipitin line with ovine LH. The latter line merged with one of the 5 precipitin lines given by FSH. Bovine LH gave two precipitin lines with the antiserum, one of which merged with the line appearing between ovine FSH and the antiserum. Ram serum gave a dense precipitin band and a separate precipitin line. One of the two precipitin lines given by bovine LH merged into this dense band. Porcine LH did not react with the antiserum. Sheep serum gave three distinct precipitin lines which merged with three of the five precipitin lines between ovine FSH and the antiserum. The antiserum to ovine FSH did not give any precipitin line with human and porcine FSH and LH, HCG, PMS and ovine luteotrophic hormone or prolactin (LTH).

These results indicated that the FSH preparation contained antigens which were common to sheep serum. Further, one of the antigens in ovine FSH which was common to the blood serum was also common to ovine and bovine LH. In order to determine whether the serum

<sup>1</sup> S. J. SEGAL, L. NIU, and S. HAKIM, *Proc. 1st intern. Congr. Endocrinol.*, Copenhagen (1960), p. 1093.

<sup>2</sup> S. J. SEGAL, K. A. LAURENCE, M. PERLBACHS, and SAFIA HAKIM, *Proc. 3rd intern. Symp. comp. Endocrinol.*, Japan (1961).

<sup>3</sup> SHANTA S. RAO and S. K. SHAHANI, *Immunology* 4, 1 (1961).

<sup>4</sup> O. OUCHTERLONY, *Acta path. microbiol. scand.* 25, 186 (1949).

<sup>5</sup> P. GRABAR, *Methods in Biochemical Analysis* 7, 1 (1959).

contaminant could be selectively removed, the FSH antiserum was absorbed with sheep serum and then tested against the various gonadotrophins (Figure 2). The absorbed antiserum was placed in the centre well and around it were placed ovine FSH, ovine LH, bovine LH, normal rabbit serum, sheep serum and unabsorbed antiserum. The unabsorbed antiserum was placed between ovine FSH and sheep serum for reasons already described<sup>3</sup>.

The results showed that ovine FSH gave two precipitin lines with the absorbed antiserum and five with the unabsorbed antiserum. Three of the five precipitin lines given by FSH which were removed by absorption were evidently due to the antigens the FSH had in common with blood serum. These three antigens were also common to the sheep serum as observed in Figure 2.

The antigenic analysis of ovine FSH and a study of the common antigens it has with ovine LH, serum, and bovine LH was also carried out by the immuno-electrophoretic technique (Figure 3). The immuno-electrophoretic plate contained seven reservoirs for the different antigens. These

were filled with bovine LH, ovine LH, sheep serum, ovine FSH, sheep serum, ovine LH and bovine LH. The top four antisera reservoirs were filled with absorbed antiserum and the bottom four with unabsorbed antiserum as indicated in the Figure.

The results indicated that the antigen ovine FSH had in common with ovine LH, ovine serum and bovine LH was

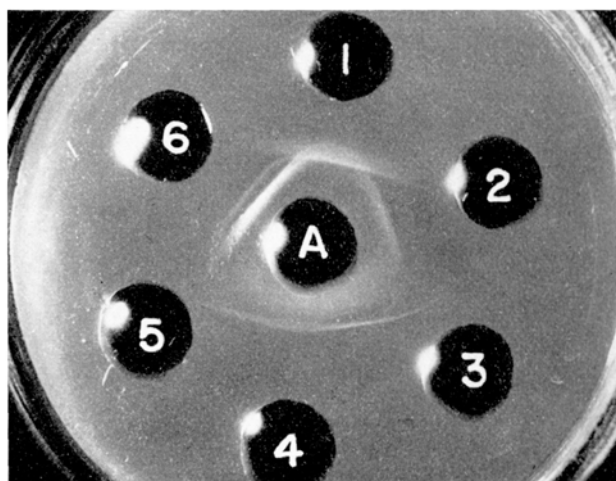


Fig. 1. Ouchterlony gel diffusion plate with rabbit antiserum to FSH. A, Rabbit antiserum to ovine FSH. 1, Ovine FSH. 2, Ovine LH. 3, Bovine LH. 4, Ram serum. 5, Porcine LH. 6, Sheep serum.

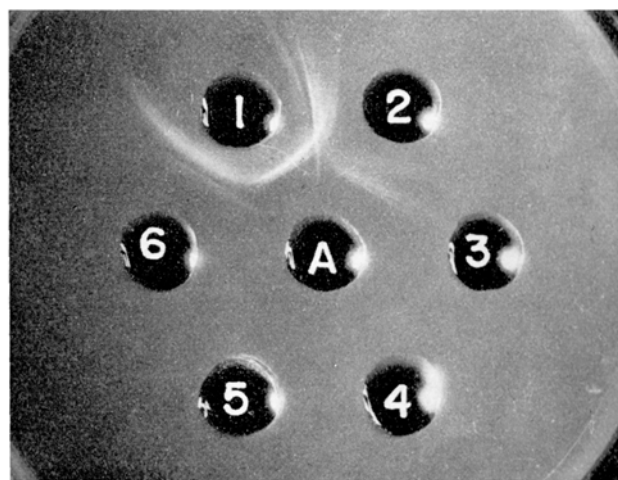


Fig. 2. Ouchterlony gel diffusion plate with absorbed FSH antiserum. - A, Rabbit antiserum to ovine FSH absorbed with sheep serum. 1, Rabbit antiserum to ovine FSH. 2, Ovine FSH. 3, Ovine LH. 4, Bovine LH. 5, Normal rabbit serum. 6, Sheep serum.

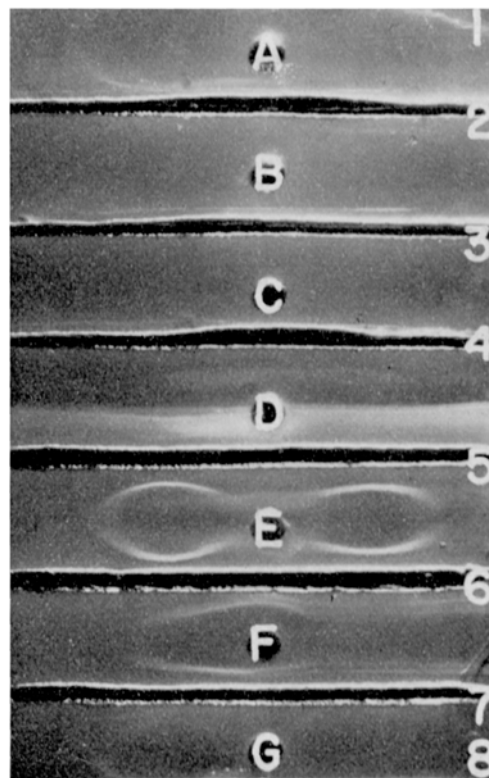


Fig. 3. Immuno-electrophoresis of absorbed and unabsorbed FSH antiserum. - Antiserum wells: 1, 2, 3, 4, Absorbed FSH antiserum. 5, 6, 7, 8, Unabsorbed FSH antiserum. - Antigen wells: A, Bovine LH. B, Ovine LH. C, Sheep serum. D, Ovine FSH. E, Sheep serum. F, Ovine LH. G, Bovine LH.

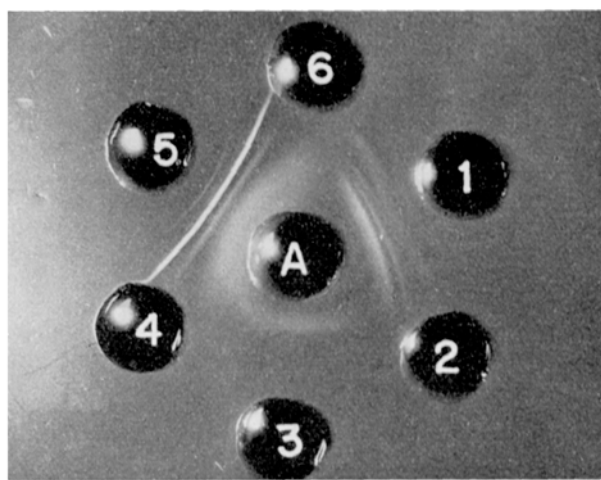


Fig. 4. Ouchterlony gel-diffusion plate with rabbit antiserum to sheep serum. - A, Rabbit antiserum to sheep serum. 1, Sheep serum. 2, Ovine FSH. 3, Ovine LH. 4, Bovine LH. 5, Ram serum. 6, Porcine LH.

completely removed by absorbing the antiserum to FSH with blood serum. Bovine LH gave two precipitin lines with unabsorbed and a single precipitin line with absorbed antiserum, indicating that one of the antigen was specific only to the bovine hormone preparation.

Experiments were also carried out with a specific rabbit antiserum obtained with sheep serum as the antigen. In the Ouchterlony plate the antiserum was placed against sheep serum, ovine FSH, ovine LH, bovine LH, ram serum and porcine LH to study the common antigens these hormone preparations have in common with sheep serum (Figure 4). The results indicated that sheep and ram sera had as many as 6 to 8 antigens of which one was common to ovine FSH and LH and bovine LH. Porcine LH did not show any reaction.

The studies reported here have shown the presence of both LH and sheep serum contaminants in ovine FSH. A common precipitin line between antiserum to sheep serum and FSH, LH and sheep serum (Figure 4) and also the common precipitin line between antiserum to sheep FSH, LH, FSH and sheep serum (Figure 1) strongly suggests the LH contaminant observed is also common to sheep serum. The observation that absorption of the antiserum to FSH with sheep serum selectively removes the antigen common to ovine FSH, LH and serum indicates that normal sheep and ram serum have LH activity which can be demonstrated in the agar diffusion test. Our results, as well as the observation of SEGAL et al.<sup>1,2</sup> that the specific antiserum to LH removes the LH contaminant in FSH and also gives a negative Weaver Finch test, strongly suggest that one of the antigen in ovine serum common to ovine FSH is due to LH. It would be worth while to prove that this particular contaminant is only due to LH. Biological experiments are under progress to prove this.

SEGAL et al.<sup>1</sup> have shown that removal of LH activity from ovine FSH causes loss of gonadotrophic potency of the material as studied by mouse uterine weight assay. It would be interesting to find out whether absorption of ovine FSH with antiserum to sheep serum would cause a

similar loss of gonadotrophic activity of ovine FSH. Work now under progress will be presented in a later publication. The results reported here also indicate that ovine LH does not have antigens in common with ovine LTH. Preliminary experiments have indicated that the antiserum to sheep serum causes inhibition of testicular weights and those of the accessory sex organs in immature male rats. The results of the *in vivo* experiments and other immunological work will be reported in a detailed publication<sup>6</sup>.

**Résumé.** L'hormone folliculaire ovine (FSH) purifiée révèle la présence d'antigènes d'hormone lutéinaire (LH) et de ceux du sérum ovin. L'antigène LH fait partie de ces derniers. L'absorption des antigènes d'antisérum de l'FSH avec le sérum ovin, élimine l'antigène qui accompagne ceux de l'hormone folliculaire ovine, de l'hormone lutéinaire et du sérum ovin. Ce fait suggère que le sérum ovin agit comme l'hormone lutéinaire. L'antisérum d'hormone lutéinaire est capable d'éliminer l'antigène d'hormone lutéinaire et les antigènes d'hormone folliculaire ovine. Cela signifie que l'un des antigènes du sérum ovin qui est présent aussi dans l'hormone folliculaire ovine, provient de l'hormone lutéinaire. Nos études en cours tendent à vérifier ce fait.

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<sup>6</sup> Ovine FSH and LH used in these studies were a gift from the Endocrinology Study Section, National Institute of Health. Human, bovine and porcine FSH and LH were a gift from Dr. A. E. WILHELM, Emory University, Atlanta (Georgia). We are grateful to them for making these hormones available. It is a pleasure to acknowledge the help and encouragement given by the Director, Dr. V. R. KHANOLKAR. – This investigation is supported by a grant from the Indian Council of Medical Research.

### Histochemistry of the Cytoplasmic Droplet in the Mammalian Spermatozoon

The cytoplasmic droplet invests the neck of young mammalian spermatozoon. By employing light and phase-contrast microscopy and classical methods of technique, some workers have described certain inclusions in the cytoplasmic droplet. According to GATENBY and WOODGER<sup>1</sup>, the cytoplasmic droplet in the spermatozoon of *Cavia* contains a number of 'argentophil platelets or rods', which impregnate exactly like the 'Golgi apparatus' of younger spermatogenic cells. Later GATENBY and WIGODER<sup>2</sup> show its 'Golgi apparatus' as a reticulum. But SHARMA et al.<sup>3</sup> state that in *Cavia* its 'Golgi elements' are in the form of granules. Late Miss DHILLON (quoted from NATH<sup>4</sup>), working with the phase-contrast microscope, demonstrated 'Golgi elements' in the form of granules in the cytoplasmic droplet of spermatozoa of rats. GRESSON<sup>5</sup>, while reviewing his previous observations on the spermatogenesis of mammals, states that 'a number of granules and irregularly shaped bodies' are present in it. NATH<sup>4</sup>, however, is of the opinion that the 'irregularly shaped bodies' of GRESSON are artefacts. From the previous literature<sup>6</sup> it seems that no attempt has been made to study the histochemistry of its cytoplasmic inclusions.

The cytoplasmic droplet appears to play some significant role in the physiology of mammalian spermatozoon. Therefore, it was considered useful to describe here the results of a study of its histochemistry in certain mammals. For this investigation, the testicular material of the goat, sheep and buffalo was used. It was treated with various histochemical techniques<sup>7</sup>. Some classical 'Golgi' techniques, such as those of AOYAMA and KOLATCHEV, were also employed.

As the spermatid of the goat, sheep and buffalo differentiates into spermatozoon, most of its cytoplasm and cytoplasmic inclusions are gradually sloughed off through the posterior regions of the spermatozoon tail. However,

<sup>1</sup> J. B. GATENBY and J. H. WOODGER, *Quart. J. micr. Sci.* **65**, 265 (1921).

<sup>2</sup> J. B. GATENBY and S. B. WIGODER, *Proc. Roy. Soc. B* **104**, 471 (1929).

<sup>3</sup> G. P. SHARMA, G. C. CHAUDHURI, and V. S. SATTEE, *Res. Bull. Panjab Univ.* **38**, 157 (1953).

<sup>4</sup> V. NATH, *Res. Bull. Panjab Univ.* **95**, 1 (1957).

<sup>5</sup> R. A. R. GRESSON, *Cellule* **54**, 81 (1951).

<sup>6</sup> M. W. H. BISHOP and A. WALTON, in MARSHALL'S *Physiology of Reproduction* (ed. by A. S. PARKES, 1960), 3rd edition.

<sup>7</sup> S. S. GURAYA, *Cellule* **62**, 95 (1961).