by one set of pituitary halves (Figure 1) and 24.2 ± 1.2 and 24.7 ± 1.5 ng/h by another set (Figure 2). It is apparent that when the conditions are identical, the amount of prolactin released by one set of pituitary halves is essentially the same as that released by the set of opposite halves. During the second hour, when plasma was substituted for medium 199, the rates of release of prolactin by pituitary halves incubated in peripheral plasma were 21.2 ± 1.2 (Figure 1) and 23.6 ± 1.2 ng/h (Figure 2), which is essentially the same as that released in the first hour when medium 199 was the incubation fluid. The pituitary halves incubated in stalk plasma from untreated animals released 18.7 ± 1.3 ng/h (Figure 1). The halves incubated in stalk plasma from dopamine-treated rats released 5.9 ± 0.6 ng/h or $^1/_4$ as much prolactin as their opposite halves which were incubated in peripheral plasma (Figure 2).

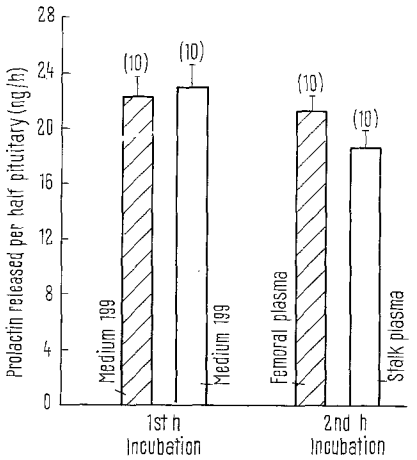

Fig. 1. The effect of hypophysial stalk plasma from untreated rats on prolactin release by rat hemipituitaries in vitro. The number of hemipituitaries is shown in parentheses. The vertical bar represents the standard error.

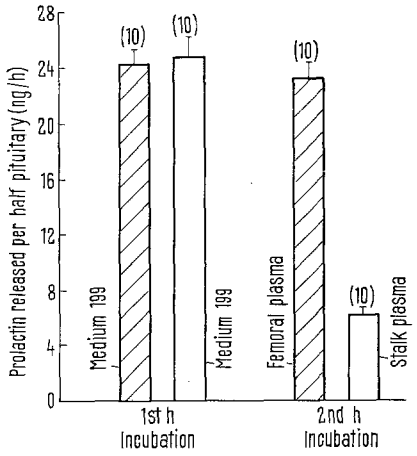

Fig. 2. The effect of hypophysial stalk plasma from dopamine-treated rats on prolactin release by rat hemipituitaries in vitro. The number of hemipituitaries is shown in parentheses. The vertical bar represents the standard error.

An analysis of these data using the *t*-test for paired samples[30] revealed that the pituitary halves incubated in stalk plasma from untreated animals and from dopamine-treated animals released significantly less prolactin than their opposite halves which were incubated in peripheral plasma ($P < 0.001$). In addition, pituitary halves incubated in stalk plasma from dopamine-treated rats released significantly less prolactin than pituitary halves incubated in stalk plasma from untreated rats ($P < 0.001$).

These observations show that pituitary stalk plasma in the rat contains prolactin-inhibiting activity and the activity in stalk plasma is increased when dopamine is injected into the third ventricle of the brain. The prolactin-inhibiting activity is attributed to PIF, the release of which may be regulated by a dopaminergic mechanism[31].

Résumé. Des moitiés d'hypophyse incubées dans du plasma de la tige hypophysaire sécrètent moins de prolactine que les moitiés complémentaires incubées dans du plasma de sang périphérique. Les glandes incubées dans du plasma de la tige hypophysaire de rats traités à la dopamine sécrètent moins de prolactine que celles incubées dans le plasma des rats témoins. L'activité inhibitrice de la prolactine dans le plasma de la tige hypophysaire peut être le résultat d'un facteur particulier, et la sécrétion de ce facteur serait gouvernée par un mécanisme «dopaminergique».

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Indication of an 'Insulin Like' Factor in the Pancreatic Tissue of the River Lamprey *Lampetra fluviatilis* (L.)

The homology between the pancreatic tissue of larval and adult lampreys and the islet tissue of the higher vertebrates first suggested by Cotronei[1], Keibel[2], and Boenig[3] has since been confirmed by both histological and experimental studies[4–15]. In the ammocoete larva of *Lampetra planeri*, a non-parasitic species which does not feed after metamorphosis, Barrington[4] showed that the secretory activity of the pancreatic tissue increased after glucose loading and that removal of the follicles by cautery increased blood sugar levels. Boenig[3] and Sterba[6] suggested that in this species the pancreas of the adult is degenerate and functionless. However, experimental studies by Ermisch[7–8] in which he showed an extract of both the cordal and cranial cords of adult

L. planeri produced a hypoglycaemic response in the mouse, indicates that this is not the case.

In the parasitic lamprey species it has been demonstrated that injection of mammalian insulin results in a reduction in blood sugar levels in *Lampetra fluviatilis*[9-10] but in the Southern hemisphere form, *Geotria australis*[16], no hypoglycaemic effect was detected when extracts of ammocoete tissues were injected into rabbits.

Material and methods. Adult river lampreys were killed by decapitation and the cranial pancreatic cords removed prior to fixation in Bouin's fluid or formal saline for histological observation or storing at −18 °C. Sections were differentially stained in Heidenhains Azan and for a specific insulin identification with N.N[1] – diethylpseudoisocyanin chloride following oxidation with performic acid, or acid permangenate[17]. An extraction of the tissue was made according to the procedure of FALKNER and MATTY[18] and later by the technique of TAYLOR[19] which gave an extract less contaminated by non insulin protein constituents. The extracts obtained were processed in three ways: a) Chromatographed by the method of FENTON[20] and stained by N,N[1]-diethylpseudoiscyanin. b) Injected i.p. into adult lampreys after which their blood glucose concentrations were determined at varying periods of time by the glucose oxidase method (Clinton reagents). c) Injected into two previously starved rabbits and their blood glucose levels determined by the glucose oxidase method at intervals over a period of $2^1/_2$ h.

Results. As previously demonstrated by BARRINGTON[5] the pancreatic cords showed one cell type on differential staining. Staining with pseudoisocyanin gave a typical reddish purple colouration to the active B cells, indicative of the presence of an insulin like molecule.

The chromatogrammed extract produced a band of protein material running at the same Rf value as that of ox-insulin standards. The amount of this material present was too small to be accurately analyzed spectrophotometrically. However, on staining with pseudoisocyanin and observation under longwave UV-radiation a positive fluorescing spot indicative of insulin was obtained.

After injection of 0.05 ml of pancreatic extract equivalent to 0.0036 g of fresh cord tissue a significant hypoglycaemic effect was shown in the lampreys.

The fall in blood glucose concentration was significantly different from that of the normal fasting level (p 0.001) and is similar to that found on injection of insulin by BENTLEY and FOLLETT[9].

Injection of similar extracts into 2 previously starved rabbits produced a marked hypoglycaemic effect followed by a rapid return to normal levels (Figure). The effect produced by both extracts was significantly different from the controls and that of the second extract in particular is similar to that found on injection of mammalian insulin[21].

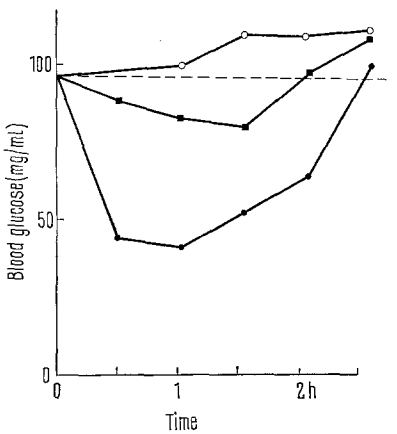
Discussion. Observations of BENTLEY and FOLLETT[9] suggest an efficient gluconeogenesis to meet the metabolic requirements in the migratory phase of *L. fluviatilis*.

The presence of an insulin like factor in *L. fluviatilis* indicated both by the hypoglycaemic response to mammalian insulin and by the regulation of blood sugar values following glucose injection[9], has been confirmed by the experiments reported here. The hypoglycaemic effect following injection of mammalian insulins into lampreys is similar to that observed after injection of homologous lamprey pancreatic extracts. Similarly the hypoglycaemic response of the rabbit to lamprey extracts is comparable with that obtained with mammalian insulin.

The positive reaction to pseudoisocyanin both of histological sections and chromatographic extracts is highly specific for the detection of insulin.

The blood glucose concentration of *Lampetra fluviatilis* at intervals following the injection of pancreatic extract

Time (h)	Average blood glucose (mg/100 ml)	S.E. of the mean	No. of animals
0	20.67	±4.52	6
1	36.33	±7.25	4
2	28.36	±7.40	3
3	17.57	±5.17	3
4	10.34	±3.81	4
5	6.93	±2.57	4



Effect of lamprey pancreatic extracts on the blood glucose levels of two previously starved rabbits. ○—○, controls injected with saline; ■—■, injection of 0.4 ml pancreatic extract in 0.9% saline (0.036 g tissue); ●—●, injection of 0.46 ml pancreatic extract in 0.9% saline (0.033 g tissue).

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Only proteins characterized by at least 2 closely neighbouring-SO_3 groups, about 4–5 Å apart react metachromatically. Such a configuration of groups occurs in the A chain of the insulin molecule after oxidation of the –SH groups.

The difference in the time of response of lampreys and rabbits supports the view of BERN and NANDI[22], that the response to insulin and the restoration of altered blood sugar levels to normal values is much slower in poikilotherms than in homiotherms.

FALKNER and WILSON[23] found that insulins from poikilothermic species the daddy sculpin (*Cottus scorpius*) and the hagfish (*Myxine glutinosa*) were effective in homologous species at lower doses than those required when ox and cod insulin were used, thus indicating a degree of species specificity. Similarly, although the lamprey 'insulin like' factor is effective in mammalian species it is probable that the molecules are not identical. ERMISH[8] suggested that this was because that portion of the insulin molecule producing its immunological specificity was different from that responsible for its physiological characteristics.

Résumé. L'existence d'un facteur pseudo-isocyanine positif semblable à l'insuline est constatée dans les coupes histologiques et dans les extraits chromotogrammés des cordons cellulaires du pancréas de *Lampetra fluviatilis*. En outre, l'extrait acide de ces cordons, injecté au lapin et la lamproie produit un effet hypoglycémique significatif.

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Pineal Inhibition of Compensatory Testicular Enlargement in Light-Deprived Hamsters[1]

Recently, many studies have been performed to determine the influence of the pineal gland or pineal substances on compensatory enlargement of the remaining gonad after unilateral castration[2–6]. The majority of these studies utilized the albino rat as the experimental animal. Because the pineal gland (epiphysis cerebri) of the golden hamster (*Mesocricetus auratus*) exhibits a strong antigonadotropic influence[7–8], the author decided to test the ability of this organ to inhibit compensatory testicular enlargement in this species.

Materials and methods. Since in adult male golden hamsters unilateral gonadectomy usually does not cause a significant hypertrophic response of the remaining testis, the following experimental design was used. 30 adult male hamsters, weighing between 94 and 115 g, were blinded by bilateral orbital enucleation. This procedure characteristically leads to total gonadal involution within 8 weeks; the atrophic response of the gonads is a result of an activated pineal since its removal prevents the involution[9]. Thus, whereas normal and blinded pinealectomized have testes weighing approximately 3000 mg, if animals are blinded only, the gonads weigh about 400 mg within 2 months. After 8 weeks the 30 blinded hamsters were divided into 3 groups. The first group (11 animals) was unilaterally castrated and sham pinealectomized; the removed testis was weighed to the nearest mg. The second group (10 animals) was unilaterally castrated and pinealectomized; the weights of the excised testes were again recorded. The third group (9 animals) was subjected to pinealectomy only and the weight of 1 testis was estimated in the following manner. The testis was exposed through an incision in the lower abdominal wall and its size was compared to a similarly atrophic testis of known weight. In this manner the weight of the exposed testis could be accurately predicted; previous experience had shown that, using this procedure, it was relatively easy to estimate the weight within ± 10 mg.

After the operations the animals from each group were housed, 4–6 per cage, and were allowed free access to food and water. The animals were maintained under these conditions for 21 days. At necropsy, the remaining testis from each of the hamsters of the first two experimental groups and 1 testis from each animal in group 3 were weighed to the nearest mg. All testes were retained for histological study.

Results and discussion. Unilateral gonadectomy in blinded hamsters failed to elicit a significant hypertrophic response within 3 weeks (Figure). In fact, the remaining testes in these animals exhibited, on the average, only a 2% increase in weight. By comparison, unilaterally castrated hamsters that were also pinealectomized showed a highly significant ($p < 0.001$) incremental change in the size of the remaining gonad; in this group there was a 105% increase in the size of the remaining testes. By comparing the initial and final testicular weights, it was obvious that in each of the 10 animals in this group, the remaining testes underwent some hypertrophy. Pinealectomy alone caused a slight (31%), but statistically insignificant, enlargement of the gonads. All testes examined were histologically atrophic.

The results clearly indicate that the activated pineal gland of the blinded hamster is capable of suppressing compensatory testicular enlargement since the removal of this organ allows for a substantial growth of the intact gonad after unilateral castration. These findings agree with studies in the rat where the injection of pineal

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