food source, or inability to find a mate. When the microhabitat is no longer favorable, the macropterous morphotypes are produced and the winged progeny can seek other favorable habitats. To an unusual degree, this fly has evolved toward an ideal mode of life that takes maximum advantage of a favorable and stable larval habitat without sacrificing capacity for migration from deteriorating habitats (*6*), dispersal, or gene flow. I suspect that other species thought to be micropterous or apterous will also prove to have macropterous morphotypes.

Apparently the differentiation of this polymorphic organism involves the selection of one developmental pathway from two potential routes (*7*). The particular environmental switch mechanism in the *P. perniciosa* system has not been determined, but it appears to be associated with crowding, nutritional requirements, moisture requirements, or any combination of these factors acting on the early instar larvae. The environmental factor or factors would presumably act on the endocrine system of the differentiating individual (*8*). Southwood (*9*) reviewed the information available on alary polymorphism in Heteroptera, concluding that short-wingedness was a juvenile character brought about by excessive influence of the juvenile hormone. Polymorphism in *P. perniciosa* involves considerably more than the wings; in fact, almost every external adult feature is affected. Considering the extraordinary polymorphism found in both sexes of this species and its suitability for laboratory and genetic studies, *P. perniciosa* presents an exceptional laboratory model for elucidating some of the genetic and environmental parameters relating to polymorphism.

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1. The term micropterous, used in the sense of Hackman [*Not. Entomol.* **44**, 73 (1964)], is convenient as applied here but inadequate in that it does not describe the extreme reduction of other external features in this morphotype.
2. Rearing methods are reported by W. Steffan, *Pac. Insects*, in press.
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5. This species is digenic—that is, progeny from a single female include both males and females; monogenic reproduction is commonly found in some other Sciaridae [C. W. Metz, *Am. Nat.* **72**, 485 (1938); H. V. Crouse, *Genetics* **45**, 1429 (1960)].
6. Southwood's discussion [*Biol. Rev. Cambridge Phil. Soc.* **37**, 171 (1962)] of facultative migration as a response by a species to early signs of deterioration of the environment provides some understanding as to the possible evolutionary significance of polymorphism in *P. perniciosa*.
7. The selection mechanism proposed by Lees [*Symp. R. Entomol. Soc. Lond.* **1**, 67 (1961)] in his studies of dual polymorphism in aphids, which suggests that the environment in some way controls the choice of alternate paths of development with nuclear genes participating in the realization of polymorphic characters, offers a possible explanation of the mechanism of differentiation in *P. perniciosa*.
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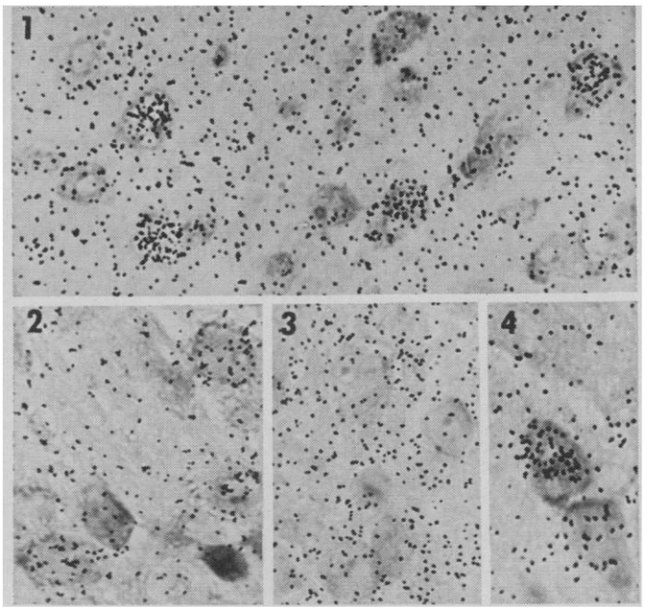
13 August 1973

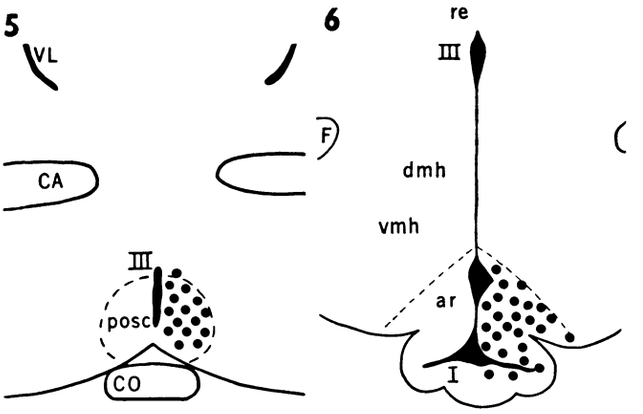
Neurons of the Hypothalamus Concentrate [^{3}H]Progesterone or Its Metabolites

Abstract. *Selective concentration of [^{3}H]progesterone or its metabolites is observed in nuclei of neurons in certain hypothalamic regions of the guinea pig 15 minutes after injection of [1,2,6,7-^{3}H]progesterone, by use of dry-mount autoradiography. Highest concentrations of progestin target neurons exist in the nucleus arcuatus, the nucleus preopticus periventricularis, and the nucleus preopticus suprachiasmaticus. Previous administration of unlabeled progesterone inhibits the nuclear concentration of radioactivity, but cortisol has no effect. Estradiol priming enhances the nuclear uptake of radioactivity. The results demonstrate the existence of progestin target sites in the hypothalamus and suggest a direct action of progestin on certain hypothalamic structures.*

Progesterone has been shown to exert both stimulatory and inhibitory effects on ovulation (*1*). Intracranial and intrapituitary implants of progestogens suggest a site of action affecting gonadotropin secretion at the level of the hypothalamus and pituitary (*2–6*). Recent studies also indicate that a facilitation of estrous behavior in female rats was induced when intracranial implants of progesterone were placed in the medial basal hypothalamus, but not when placed in the mesencephalic reticular formation (*7*). However, the precise site or sites at which progesterone acts to block ovulation and to facilitate sexual receptivity still remains controversial. In the hypothalamus, no selective accumulation of progesterone or its metabolites could be found (*8*), although in the uterus a specific progesterone binding protein has been demonstrated (*9–11*). Using the dry-mount autoradiographic technique, we have identified progesterone target cells in the uterus (*12*), similar to estrogen and androgen target cells in reproductive organs, brain, and pituitary (*13*). The present report demonstrates for the first time a selective cellular and subcellular concentration of progesterone or its metabolites in certain neurons of the hypothalamus.

Eight 35-day-old female guinea pigs of the Hartley strain, weighing about 350 g, were ovariectomized. Two guinea pigs were injected subcutaneously with 0.2 ml of sesame oil alone, and four guinea pigs were primed with 10 μg of estradiol-17β, dissolved in sesame oil, daily for 5 days. The two nonprimed animals and the four primed with estrogen were injected intravenously with 1 μg per 100 g of body weight of [1,2,6,7-^{3}H]progesterone, specific activity 110 c/mmole, dissolved in 10 percent ethanol in isotonic saline. To show the specificity of progestin localization, 5 minutes prior to the injection of [^{3}H]progesterone, one estrogen-primed guinea pig was injected intravenously with either 500 μg of progesterone or 1 mg of cortisol dissolved in 50 percent ethanol in saline. The animals were decapitated after 15 minutes, since at this time interval nuclear uptake of radioactivity was optimal in progesterone target tissues (*12*). The hypothalamus was excised, mounted on a tissue holder, and frozen in −180°C liquefied propane. Serial frozen sections were cut in a wide range cryostat (Harris Mfg. Co., Cambridge, Mass.) and freeze-dried with a Cryo-pump (Thermovac Industries, Copiague, N.Y.). The freeze-dried sections were dry-mounted on slides coated with desiccated emulsion (Kodak NTB 3). After autoradiographic exposure for 2 to 3 months, the slides were photographically processed and stained with methyl green–pyronin. Autoradiograms of diaphragm prepared in the same way served as controls. The dry-mount autoradiographic technique has been described (*14*).





Figs. 1 to 4. Autoradiograms of the arcuate nucleus showing nuclear concentration of radioactivity in certain neurons. Prepared 15 minutes after intravenous injection of [^{3}H]progesterone into 35-day ovariectomized guinea pigs. Exposure time, 60 days; thickness, 4 μm; magnification, $\times$ 810. Stained with methyl green–pyronin. Fig. 1. After priming with estradiol-17β nuclear concentration of radioactivity is increased. Fig. 2. Without priming with estradiol-17β, only comparatively weak labeling of neurons is visible. Fig. 3. After previous administration of progesterone, nuclear concentration of radioactivity is inhibited. Fig. 4. Previous administration of cortisol does not interfere with nuclear uptake of radioactivity. Figs. 5 and 6. Schematic drawings of frontal sections of guinea pig hypothalamus showing accumulation of progestin target neurons (black dots, right half) in the anterior preoptic region (Fig. 5) and central hypothalamus (Fig. 6). Prepared after serial-section autoradiograms obtained 15 minutes after [^{3}H]progesterone injection. Designation of structures: *ar*, nucleus arcuatus; *CA*, commissura anterior; *CO*, chiasma opticum; *dmh*, nucleus dorsomedialis hypothalami; *F*, fornix; *posc*, nucleus preopticus suprachiasmaticus; *re*, nucleus reuniens; *III*, third ventricle; *VL*, lateral ventricle; *vmh*, nucleus ventromedialis hypothalami; and *I*, infundibulum.

Autoradiograms of the hypothalamus in estrogen-primed ovariectomized guinea pigs show concentration of radioactivity in nuclei of certain neurons (Fig. 1), in addition to a general distribution of silver grains seen over the cytoplasm and extracellular space. Glial cells as well as ependymal cells of the third ventricle do not show retention of radioactivity. When ovariectomized animals not primed with estrogen are compared with the estrogen-primed animals, only a weak neuronal nuclear accumulation of radioactivity is observed (Fig. 2) in the nonprimed group (*15*). The estrogen-priming effect on increase of nuclear concentration of radioactivity in certain hypothalamic neurons may be attributable to an increase of progestin binding protein as reported for rat, guinea pig, and rabbit uteri (*9, 11, 16*). Administration of 500 μg of progesterone 5 minutes prior to the injection of tritium-labeled progesterone inhibits nuclear concentration of radioactivity in neurons of this region (Fig. 3), whereas 1 mg of cortisol does not have such an effect (Fig. 4) (*15*). The results of the competition experiments with progesterone and cortisol indicate that the radioactivity in the hypothalamus is the labeled progesterone or its metabolites, or both.

The distribution of radioactively labeled cells in the anterior preoptic and central hypothalamic region is depicted in Figs. 5 and 6. Neurons of the nucleus arcuatus (Figs. 1 and 6) concentrate radioactivity from its frontal to caudal extremity. Radioactively labeled neurons are most frequent in its caudal part which borders the infundibular recess of the third ventricle. A few neurons of the nucleus ventromedialis and nucleus premammillaris ventralis are also labeled. In addition to this, neurons of nucleus periventricularis preopticus, bordered anteriorly by the nucleus tractus diagonalis and posteriorly by the nucleus suprachiasmaticus, as well as neurons of nucleus preopticus suprachiasmaticus (Fig. 5), show nuclear concentration of radioactivity. Neurons of the nucleus preopticus medialis, nucleus suprachiasmaticus, nucleus supraopticus, nucleus paraventricularis, area hypothalamica anterior, nucleus dorsomedialis, nucleus ventromedialis, pars lateralis, nucleus premammillaris dorsalis, area hypothalamica posterior, and the nuclei of the mammillary body do not show concentration of radioactivity.

The results obtained by autoradiography demonstrate a selective nuclear concentration of progesterone or its metabolites in certain neurons of the brain, thus providing evidence for the existence of progestin target sites in the hypothalamus. This is consistent with the finding of intrahypothalamic implants of medroxyprogesterone acetate in the arcuate nucleus of the guinea pig, which prevent ovulation in 50 percent of the cases, whereas implants in other regions failed to do so (*4*). In the rat, progestin implants in the hypothalamus have been shown to inhibit ovulation, as they do in the guinea pig (*2*). Furthermore, our results support the observation of facilitatory effects of progesterone on medial basal hypothalamic neurons, based on electrophysiological evidence (*17*) as well as on behavioral responses (*7*).

Results obtained by use of the autoradiographic technique, in connection with reports cited from the literature on the effects of progestin, suggest that progesterone can act directly on the hypothalamus, as has also been shown for estrogen and androgen. The data also suggest that nuclear binding of progesterone in hypothalamic neurons is necessary for its central hormonal action.

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15. In the quantitative evaluation of autoradiograms, the average number (mean ± standard error) of silver grains in nuclei and cytoplasm of neurons of the arcuate nucleus in estrogen-primed guinea pig is 35.60 ± 2.40 and 6.1 ± 0.4; in nonprimed guinea pig, 11.30 ± 0.99 and 3.4 ± 0.5; and in guinea pig previously treated with cortisol, 33.70 ± 3.20 and 5.6 ± 0.5, respectively. When the distribution of silver grains is calculated per unit (square micrometer) of the nuclear and cytoplasmic area of the cell with labeled nuclei, and assuming the cells are round, the following counts were obtained for estrogen-primed animals: nuclei, 1.26, cytoplasm, 0.21; for nonprimed animals: nuclei, 0.40, cytoplasm, 0.12; and for animals pretreated with cortisol: nuclei, 1.20, cytoplasm, 0.19. The estimation of mean value in the nuclear and cytoplasmic concentration of silver grains is based on the counting of silver grains in 50 randomly selected labeled neurons after correcting for background.
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18. Supported by PHS grants NS 09914 and HD 05700, and by a grant from the Rockefeller Foundation to the Laboratories for Reproductive Biology, University of North Carolina, Chapel Hill. We thank Ms. Anu Turnbull for technical assistance.

20 June 1973; revised 10 September 1973

Phosphate-Induced Protein Chromatography

Abstract. *High phosphate concentration is shown to cause a large proportion of the proteins of a cell-free extract of* Escherichia coli *to bind to agarose columns to which* L*-valine is attached. With a decreasing concentration gradient of potassium phosphate, the proteins elute in relation to their solubility in concentrated ammonium sulfate. This column technique appears to provide a general tool for the purification of proteins.*

To date, only a relatively few properties of proteins have been used, advantageously, for the purification of this class of macromolecules in their native state. These techniques are largely dependent on charge, size, shape, solubility, and physical and chemical adsorption properties of these macromolecules (*1–3*). Techniques designed to exploit the hydrophobic properties of macromolecules for their purification have not been highly developed. This report describes a new technique that appears to provide a general method for the purification of proteins, which may operate primarily on the basis of the hydrophobic characteristics of proteins.

It is shown in this report that in the presence of high potassium phosphate concentrations (1.0*M*) over half of the proteins in a cell-free extract of *Escherichia coli* are retarded on agarose columns to which aliphatic substituents have been attached. It is further demonstrated that it is possible to elute and chromatograph these proteins by utilizing a decreasing potassium phosphate concentration gradient.

The soluble proteins from a dialyzed cell-free extract of *E. coli* strain K12 were placed on a column of L-valine substituted agarose in the presence of 1.0*M* potassium phosphate, *p*H 7.5. The elution profile of these proteins observed during the development of a 1.0 to 0.1*M* potassium phosphate concentration gradient is shown in Fig. 1a. About half of the proteins applied to this column chromatographed throughout the concentration gradient. In addition, a large amount of material that absorbs strongly at 260 nm (presumably polynucleotides) also eluted throughout the course of the concentration gradient (Fig. 1a). In order to determine whether this 260-nm absorbing material affected the chromatographic behavior of these proteins, a dialyzed cell-free extract of *E. coli* K12 was treated with 2 percent streptomycin sulfate to remove the nucleic acids. This dialyzed extract was chromatographed as before. This procedure removed over 90 percent of the 260-nm absorbing material from the extract, and the chromatography of the proteins was not significantly altered (Fig. 1b). The nature and chromatographic behavior of this 260-nm absorbing material requires investigation.

To determine whether a significant amount of protein remained on the column after completion of the decreasing gradient, the column was washed with several column volumes of a high-ionic-strength solution of a mild chaotropic agent (1.0*M* potassium bromide in 0.05*M* potassium phosphate, *p*H 7.5). That this treatment did not further remove any protein from the column suggests that retention of the proteins on the column due to ion exchange at the low potassium phosphate concentrations is unimportant. However, a measurable amount of denatured protein was removed from the column by 0.1*N* sodium hydroxide (Fig. 1b).

Figure 1c shows that the proteins from these extracts elute within the void volume of an unsubstituted agarose column developed under the same decreasing potassium phosphate concentration gradient conditions.

Since phosphate and sulfate ions are structure-forming ions, which are known to decrease the solubility of proteins and to stabilize hydrophobic bonds between nonpolar molecules (*4*), we attempted to establish a relationship between the solubility of the proteins of an *E. coli* cell-free extract in concentrated ammonium sulfate and the position at which these proteins appear in a decreasing potassium phosphate concentration gradient. Accordingly, three nucleic acid–free ammonium sulfate fractions (0 to 35 percent, 35 to 50 percent, 50 to 65 percent of saturation) of a cell-free extract of *E. coli* K12 were dialyzed free of ammonium sulfate and placed on separate columns. The 0 to 35 percent fraction, which contained the proteins that were least soluble and, therefore, presumably the most hydrophobic (*4*), were chromatographed across the entire decreasing concentration gradient (Fig. 1d). A greater proportion of these proteins was eluted at the lower potassium phosphate concentrations than was observed for the 35 to 50 percent fraction (Fig. 1e). The most soluble, least hydrophobic, proteins, which are in the