of the eye color substances in the crushed pupae. The decline in effect in older test larvae is explained by the fact that they take in less food before puparium formation than do younger larvae.

Tests have shown that somewhat stronger effects are obtained on feeding crushed pupae if the material to be fed is not allowed to come in contact with the agar. The active substances are known to diffuse into agar where presumably they are less available to the larvae.

Active water solutions of the two substances under consideration, prepared and made available by Dr. Edward L. Tatum, have given positive reactions when mixed with yeast and fed as described above.

Summary. The hormone-like v^+ and cn^+ substances concerned in the development of eye colors in Drosophila produce their characteristic reactions when administered with the food of test larvae. In this way they are comparable with certain hormones of the vertebrates, for example, thyroxin.

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Electrophoresis of Prolan.*

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No gonadotropic substance has ever been isolated in pure form. Nevertheless, an empirical idea of the chemical nature of these substances has been deduced from their solubility, their failure to diffuse through membranes, and their inactivation by certain reagents. The gist of these experiments[1-4] is that the gonadotropic hormones are protein-like in character and perhaps are muco-proteins similar to certain enzymes.

The object of the present work is to bring a new technique, namely that of electrophoresis, to bear upon the problem of the characterization of gonadotropic substances. The behavior of an amphoteric substance such as a protein in an electrophoretic cell

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1 Fischer, F. G., and Ertel, L., *Z. physiol. Chem.*, 1931, **202**, 83.

2 Haurowitz, F., Reiss, M., and Balint, J., *Ibid.*, 1933, **222**, 46.

3 Bischoff and Long, *J. Biol. Chem.*, 1936, **116**, 285.

4 V. Euler, H., and Zondek, B., *Chem. Abs.*, 1934, **28**, 4085; *Skand. Arch. Physiol.*, 1934, **68**, 232.

can, in favorable cases, reveal the isoelectric point of the compound. This is probably the only definite, measurable property of a protein which may be determined by physical means in a crude mixture.

Prolan, the gonadotropic principle of pregnancy urine, was selected as the first of a series of substances to be investigated. A sample of so-called alcohol prolan, obtained by precipitation with alcohol, was used as a preparation least likely to have undergone chemical change in isolation. A second preparation was made by the brucine method of Katzman and Doisy.[5]

Crude alcohol prolan was prepared from late pregnancy urine by precipitation with 18 volumes of alcohol. The dried powder was assayed by the method described below. Twenty milligrams gave ovaries weighing 40 mg. Brucine prolan prepared by Katzman and Doisy's method,[5] was similarly assayed; 16 mg. gave 24 mg. ovaries.

The electrophoresis apparatus was of the conventional U-type.[6] The central chamber was of 12 mm. bore and held 20 cc. The adjoining anode and cathode chambers held 10 cc. each. Electrodes were of sheet copper and set into 20 cc. cups sealed to the bottom of 250 cc. flasks. The anode was packed with rock salt and the cathode with crystals of copper sulfate. The current source was the 110 volt D.C. line without resistance.

Alcohol Prolan. Four hundred milligrams was dissolved in 20 cc. of water, and 5 cc. of molar sodium acetate-acetic acid buffer solution was added. The material became completely soluble in a few minutes. The pH was measured with a quinhydrone electrode. It was placed in the central chamber, and after rinsing, the anode and cathode chambers were filled with M/15 buffer solution of the same pH, and the electrophoresis was begun. After 18 hours the anodic and cathodic fractions were collected separately and neutralized. The pH of the central chamber was checked, and in no case was the drift more than 0.08 of a pH unit. Mean pH values are given in Table I. The procedure was repeated with fresh material and the 2 samples were pooled.

Four rats each were used for assaying the contents of the cathode and of the anode chambers at each pH value. The animals were immature female rats, 21 days old at the time the injections were begun. Each rat received a total quantity of 4.5 cc., 0.5 cc. of this being injected twice daily for 4.5 days, with autopsy on the morning of the sixth day.

[5] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1934, **107**, 513.

[6] Hartman, R. J., and Cheng, L. T., *J. Phys. Chem.*, 1936, **40**, 454.

TABLE I.
Electrophoresis of Alcohol Prolan.

pH	Anode			Cathode		
	Foll.	C.L.	Ut. Stim.	Foll.	C.L.	Ut. Stim.
4.91	4*	4	4	0*	0	0
4.57	4	2	4	0	0	0
3.95	3	2	4	0	0	0
3.70	3	0	4	0	0	0
3.55	1	0	2	0	0	0
3.31	0	0	0	0	0	0

*Figures indicate the number of rats showing follicular development, corpora lutea, or enlarged uterus.

The presence or absence of gonadotropic material in the anode, cathode, and in certain cases in the central chamber was determined by the ability of samples of the contents to cause development of follicles, corpora lutea, or the uterus. The weight of the ovaries of all animals showing stimulation of one or more of these structures was not much greater than that found for untreated immature animals of the same age. In Table I are recorded the number of animals showing development of corpora lutea, follicles or uterus. No attempt was made to make a study of the precise quantity of gonadotropic material which migrated from the central chamber.

Brucine Prolan. One hundred milligrams were used in each charge. Otherwise the procedure was as before. The data are recorded in Table II.

TABLE II.
Electrophoresis of Brucine Prolan.

pH	Anode			Cathode		
	Foll.	C.L.	Ut. Stim.	Foll.	C.L.	Ut. Stim.
5.8	4*	4	4	1*	0	0
5.1	4	3	4	3	1	3
4.4	4	3	4	4	2	4
4.0	4	4	4	4	4	4
3.5	4	1	4	4	1	4

*Figures indicate the number of rats showing follicular development, corpora lutea, or enlarged uterus.

The data of Table I show that with alcohol-precipitated prolan there is definite and unmistakable gonadotropic activity in the *anodic* material from pH 4.91 to pH 3.55, with a decrease in activity toward the acid side. At no pH does the hormone behave as a cation. At pH 3.55 where migration is slight, an appropriate blank experiment and analysis of the central chamber showed that the absence of activity is not attributable to acidity or to heating by the electric current. This would suggest an approach to the iso-

electric point, but as the table indicates, increase of acidity to pH 3.31 did not induce migration to the cathode although bioassay of the contents of the central chamber demonstrated considerable, but a lesser than expected amount of gonadotropic activity. This indicates that the high acidity probably is responsible for the absence of activity in the anode and cathode chambers at pH 3.31. Prolan is definitely inactivated in greater concentrations of acid. The data indicate that alcohol prolan has an isoelectric point on the acid side of pH 3.5, and probably in the neighborhood of pH 3.3. Isoelectric points in this highly acid range are characteristic of mucoproteins.[7]

An entirely different set of results was obtained from prolan prepared by the brucine technique as shown in Table II. Such material was necessarily subjected to the action of strong acids and bases, and hence had probably undergone chemical change. Gonadotropic activity was found in both *anode* and *cathode chambers* from pH 5.1 to 3.5. The data suggest that the prolan complex consists of a non-specific carrier in combination with a specific residue which continues to be biologically active after the carrier has undergone rather profound change as evidenced by shift of isoelectric point. A similar hypothesis has been advanced by Euler and Zondek,[4] and comparable electrophoretic behavior has been observed with oxytocin.[8]

Concerning the problem of the plural nature of gonadotropic hormones, we are prepared to make no allegations on the basis of our experiments. We have observed exclusive follicular development induced by specimens obtained at those pH values whereat migration was slight, but this is the usual phenomenon at low dose levels.

Summary. Alcohol prolan shows the electrokinetic behavior of a single chemical individual. The isoelectric point is more acid than pH 3.5. Brucine prolan behaves as a mixture. The physiological activity of prolan is attributed to a grouping which may survive chemical treatment drastic enough to alter the protein-like carrier.

[7] Roche, A., *Compt. rend. soc. biol.*, 1935, **120**, 1229.

[8] Das, N., Ghosh, B. N., Guha, B. D., *Z. physiol. Chem.*, 1936, **238**, 131.