acetyldopamine, with the cuticular matrix, or an enzyme, possibly one of the compounds of the complex phenoloxidase system (*7*).

C. E. SEKERIS
Physiologisch-chemisches Institut der Universität München, München, Germany

References and Notes

1. M. G. Pryor, *Comparative Biochemistry* (Academic Press, New York, 1963), vol. 4, p. 371.
2. P. Karlson, C. E. Sekeris, K. Sekeri, *Z. Physiol. Chem.* **327**, 86 (1962).
3. C. E. Sekeris and P. Karlson, *Biochim. Biophys. Acta* **62**, 103 (1962); P. Karlson and C. E. Sekeris, *Nature* **195**, 183 (1962).
4. P. Karlson and C. E. Sekeris, *Biochim. Biophys. Acta* **63**, 489 (1962).
5. G. Fraenkel and C. Hsiao, *Science* **138**, 27 (1962); **141**, 1057 (1963).
6. C. B. Cotrell, *Trans. Roy. Entomol. Soc.* **114**, 317 (1962).
7. P. Karlson and A. Schweiger, *Z. Physiol. Chem.* **323**, 199 (1961); P. Karlson and H. Liebau, *ibid.* **326**, 135 (1961).
8. I thank Frau G. Behrens for skillful technical assistance and the Deutsche Forschungsgemeinschaft for financial aid.

20 February 1964

Fertility Restoration and Its Inheritance in Cytoplasmic Male-Sterile Wheat

Abstract. *Male-sterile* Triticum aestivum *L. 'Bison,' possessing cytoplasm of* T. timopheevi *Zhuk., typically produces male-sterile progeny when pollinated with common wheat cultivars. A fertility restorer developed by transferring genes from* T. timopheevi *to* T. aestivum *produced fertile hybrids when used as the pollen parent in a cross with male-sterile Bison. Data on the* F_2 *and testcross plants indicate that two dominant genes, designated* Rf_1 *and* Rf_2*, condition fertility in wheats with* T. timopheevi *cytoplasm.*

Male sterility due to the interaction of nuclear genes with specific cytoplasm is known in many plant species (*1*). This kind of male sterility frequently makes possible mass production of crossed seed of economic species and thus permits commercial culture of vigorous hybrids.

Three cases of male sterility attributable to interactions of genes and cytoplasm have been reported in common hexaploid wheat, *Triticum aestivum* L. ($2n = 42$). The first two were produced by breeding procedures which resulted in substitution of the chromosomes of common wheat in the cytoplasm of related species of goatgrass, *Aegilops caudata* L. (*2*) and *A. ovata* L. (*3*). Common wheats used thus far as pollinators of these two male-sterile types have produced only male-sterile hybrids. Recently, a more promising sterility system was developed (*4*) by backcrossing so that the chromosomes of the common wheat Bison were substituted in the cytoplasm of *T. timopheevi* Zhuk. ($2n = 28$). Male-sterile Bison was pollinated with a hexaploid having *T. timopheevi* ancestry and fertile plants were produced (*5*). The report is concerned with the inheritance of fertility-restoring genes transferred from *T. timopheevi* to hexaploid wheat.

A hexaploid fertility restorer with *T. timopheevi* cytoplasm was developed by crossing *T. timopheevi* × *T. aestivum* 'Marquis' and then backcrossing twice to Marquis as the pollinator. The resulting F_2 generation had the pedigree *T. timopheevi* × Marquis[3] (*6*). A selected plant from this population proved to be homozygous for genes conditioning fertility in *T. timopheevi* cytoplasm. All progeny resulting from self-pollination were fully fertile. Furthermore, when male-sterile Bison was pollinated with this selected plant, seven hybrid plants were produced in which the pollen and the number of seeds developing was normal showing restoration of fertility to be completely dominant. The fertile members of the F_1 generation were self-pollinated and also backcrossed with male-sterile Bison. Plants of the resulting F_2 and testcross generations were classified according to the microscopic appearance of the pollen as normal, partially fertile, or sterile. Distribution of segregating F_2 and testcross plants into these three fertility classes is recorded in Table 1. Chi-square tests indicate that a two-factor hypothesis is in accord with the observations.

Table 1. Frequency distribution in the F_2 generation and testcross populations segregating for male sterility compared with expectations from digenic inheritance.

Pollen class	Normal	Partly fertile	Sterile
F_2, male sterile × fertility restorer			
Ratio tested	9	6	1
Observed	30	28	3
Calculated	34	23	4
Chi-square probability .41			
Testcross, male sterile × fertile F_1			
Ratio tested	1	2	1
Observed	31	46	29
Calculated	26.5	53	26.5
Chi-square probability .40			

These results can be explained by assuming that the newly-developed fertility restorer is homozygous for two dominant genes, designated Rf_1 and Rf_2, which produce normal fertility in wheats with *T. timopheevi* cytoplasm. Male-sterile Bison exemplifies the recessive genotype rf_1rf_1 rf_2rf_2 in combination with *T. timopheevi* cytoplasm. Normal Bison, essential as a pollinator in propagation of the male-sterile form, has the same recessive genotype and owes its pollen fertility to possession of *T. aestivum* cytoplasm. The heterozygote, Rf_1 rf_1 Rf_2 rf_2, produced by crossing male-sterile Bison with the new fertility restorer, proved to be fully fertile. The partially fertile segregates encountered presumably possess either the dominant gene Rf_1 or Rf_2 but not both.

Experience obtained by breeding wheat with the kind of male sterility developed in Bison has shown that it can be readily transferred to many common wheat cultivars by backcrossing. In view of its mode of inheritance, no problem is anticipated in transferring fertility restoration to other wheats as desired. Thus it is clear that the genetic requisites for experimental production of hybrid wheat are now available.

RONALD W. LIVERS
Fort Hays Branch Experiment Station, Hays, Kansas

References and Notes

1. J. R. Edwardson, *Botan. Rev.* **22**, 696 (1956); D. N. Duvick, *Econ. Botany* **13**, 167 (1959).
2. H. Kihara, *Cytologia* **16**, 177 (1951).
3. H. Fukasawa, *Wheat Information Service* No. 7, 24 (1958).
4. J. A. Wilson and W. M. Ross, *ibid.*, No. 14, 29 (1962).
5. J. W. Schmidt, V. A. Johnson, S. S. Maan, *Nebraska Expt. Sta. Quart.* **9**, 9 (1962).
6. Material kindly provided in October 1962 by J. A. Wilson, Dekalb Agricultural Association, Inc.
7. Contribution No. 187, Fort Hays Branch of the Kansas Agricultural Experiment Station.

10 February 1964

Histones from Developing Tissues of the Chicken: Heterogeneity

Abstract. *Electrophoretic analysis of the molecular heterogeneity of histones from developing and adult chicken tissues demonstrates that differentiation need not be correlated with changes in the relative proportion of individual histone molecules during development.*

One of the chief reasons for thinking that histones may be regulators of genetic activity is that histones display molecular heterogeneity, a possible provision for selective regulation of genetic activity widely thought to occur in tis-

sue differentiation (*1*). Tissues differ in the way their histones exhibit heterogeneity. During spermatogenesis in the squid and snail (*2*) histones undergo an orderly schedule of replacement which rigorously reflects the state of differentiation. "Somatic histones" of pre-spermatid stages are replaced successively by lysine-rich histones in the spermatid and then by protamines in mature sperm. However, the histones of calf tissues do not reflect the state of tissue differentiation as rigorously. Histones of the liver, kidney, and thymus are very similar when studied by ion-exchange chromatography (*3*). To understand the properties of histone heterogeneity and their relation to differentiation in the tissues of a single species, the heterogeneity of histones from adult and developing chicken tissues was investigated by starch-gel electrophoresis. The results show only minor differences between histones of different tissues and developmental stages.

Nuclei of adult and embryonic chicken tissues were prepared by homogenization and centrifugation in 2.2*M* sucrose (*4*). Approximately 2.0 g of pooled wet embryonic tissues was homogenized in a small Waring Blendor vessel with 10 volumes of cold 2.2*M* sucrose. Larger preparations were made with fresh adult tissues. The homogenates were then centrifuged (1 hour, 40,000*g*) in a Spinco preparative ultracentrifuge. The resulting reddish-brown pellets contained essentially all of the nuclei in highly purified form (60 to 90 percent of the nuclei were free of visible cytoplasmic particles). Contamination with erythrocytes was less than 5 percent. The supernatants were discarded, the interior of each tube was wiped dry, and the pellets were resuspended in cold 0.1*N* HCl to extract histones. The suspension was then centrifuged (10 min, 10,000*g*). The clear supernatant containing 90 percent of extractable histone was submitted to electrophoresis at room temperature at 2 volts per centimeter on starch gels prepared in 0.01*N* HCl, *p*H 2.1. At least .22 mg protein was applied to starch powder in chambers molded into the gel. Samples to be compared were diluted to identical protein concentration before application to the gel. After electrophoresis the gel was split horizontally into three strips and stained for protein with Buffalo Blue Black NBR; the histone patterns were then observed after destaining with Smithies' solution.

The electrophoretic patterns from developing liver show a large number of histones (Fig. 1). Adult liver has the most histones, 14, and 4-day liver has the least, 10. Judging from stain intensities, the histones with highest mobility (histones 1–3) are the least abundant and are resolved well only in the adult. The group of histones with intermediate mobility (histones 4–8) account for the majority of histone protein, with the least mobile histone group (histones 9–14) next in amount.

The changes in the histone pattern in developing liver are insignificant compared to the replacement of one kind of histone by another during squid and snail spermatogenesis (*2*). The early embryonic liver is equipped with essentially all of the histones it will ever have during development. The histone patterns give a general impression of constancy which does not rigorously reflect the course of differentiation in the liver. There is no overall trend toward increase in amount of certain histones or decrease of others. For example, histones 4 to 10 are present throughout the developmental series, and the relative amounts of these change very little. There are also several minor qualitative changes, as with histones 11 and 12, which appear to resolve from a larger band early in development. It is always possible that some of the variations in the patterns may be due to variability inherent to the electrophoresis itself.

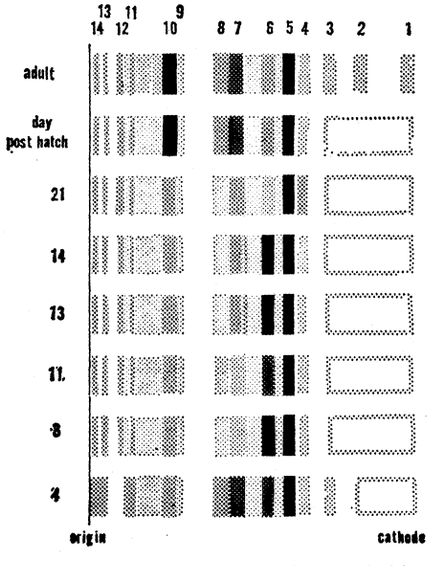

Fig. 1. Electrophoretic patterns of histones from developing chicken liver. Intensity of dotted bands represents intensity of protein staining. Areas enclosed by dotted lines represent very faint staining. Numbers along origin represent days of incubation. Numbering of histones begins with the fastest one. Histone 7 at 13 and 14 days resolves into a faster 7*a* band and a 7*b* band.

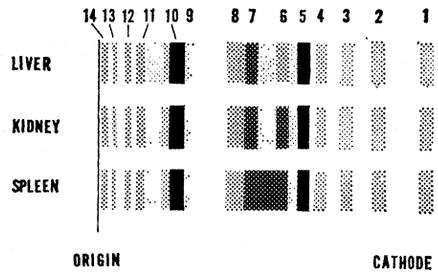

Fig. 2. Electrophoretic patterns of histones from adult chicken tissues.

Even though the liver-histone patterns from a large number of developmental stages were examined, the tissue may not have carried out functional changes radical enough for histone variations to be recognized. Therefore, histones were prepared from nuclei of mesonephros and metanephros at stages before, during, and after the period metanephros takes over excretory functions from the degenerating mesonephros. The histone patterns of metanephros and degenerating mesonephros show no major differences, and again the patterns cannot be correlated with the impressive developmental changes in these tissues. The generality of this conclusion is borne out by comparison of histones from adult liver, spleen, and kidney (Fig. 2). All three patterns are essentially identical in the number of histones and their relative amount. The only difference is that histones 6 and 7 from the spleen appear to migrate as one band.

Whether differentiation as a general process is correlated with changes in the histone complement depends upon the tissue. In the various developmental stages and tissues of the chicken investigated here, there are no rigorous changes or differences in the histone patterns which correlate with the state of differentiation. The same appears true for histones of calf tissues (*3*). The general constancy of the histones in these tissues means that the chromosomes are covered mostly with a common assemblage of histone molecules and that genetic regulation and differentiation must be accomplished in a manner largely unrelated to the relative proportion of each histone present.

However, in situations such as spermatogenesis (*2*), the replacement of

histone types can be closely correlated with the state of differentiation, and must produce major rearrangement of histone molecules within the chromosomes. In this instance, the changes in histones may be employed in a special differentiation process, perhaps taking place within the chromosomes to produce their condensed state in the mature sperm. Even so, the replacement of histones on this major scale probably lacks the genetic specificity which differentiation often displays. Such specificity may lie in mechanisms which precisely control the position of histones upon the chromosomes without regard to the relative amount of the histones (*5*, *6*).

DAVID T. LINDSAY
Department of Zoology,
University of Georgia, Athens

References and Notes

1. V. G. Allfrey, V. C. Littau, A. E. Mirsky, *Proc. Natl. Acad. Sci. U.S.* **49**, 414 (1963); R. C. Huang, J. Bonner, *ibid.* **48**, 1216 (1962); M. Izawa, V. G. Allfrey, A. E. Mirsky, *ibid.* **49**, 544 (1963); B. C. Moore, *ibid.* **50**, 1018 (1963).
2. D. P. Bloch, *J. Histochem. Cytochem.* **10**, 137 (1962); ———, H. Y. C. Hew, *J. Biophys. Biochem. Cytol.* **7**, 515 (1960).
3. C. F. Crampton, W. H. Stein, S. Moore, *J. Biol. Chem.* **225**, 363 (1957); S. Moore, in *Nucleoproteins*, (Interscience, New York, 1959), p. 77.
4. J. Chauveau, Y. Moulé, C. Rouiller, *Exptl. Cell Res.* **11**, 317 (1956).
5. D. T. Lindsay, abstract, *Proc. 16th Internat. Cong. Zool.* **2**, 243 (1963).
6. I thank Charlotte J. Kale for technical assistance and Dr. Claude Hinton for criticism. Supported by NSF grant GB-631.

2 February 1964

Interaction of Evoked Potentials of Neocortical and Hypothalamic Origin in the Amygdala

Abstract. *Evoked responses recorded from the amygdala of the cat after sequentially pairing neocortical and hypothalamic stimulation showed consistent suppression or depression of the response evoked by the test shocks, regardless of whether the cortical or subcortical site received the preceding conditioning shock. The possibility that functional interaction of neocortical and hypothalamic signals occurs in the amygdala is proved and an active inhibitory process is suggested.*

Much work on relationships between the brain and behavior in higher organisms has been focused on the interactions of the limbic system with other structures of the central nervous system. Anatomical and physiological evidence indicates close ties between the limbic system and the hypothalamus and, also, the neocortex. Hypothetically, limbic structures, such as the amygdala, can be viewed as correlating specific neocortical functions with motivational processes of hypothalamic origin (*1*). Neurophysiological details of such an interrelation at the neurophysiological level remain unspecified. The pilot experiments reported here were undertaken to define some of the neurophysiological variables involved in cortico-limbico-hypothalamic interactions.

In a series of 12 cats under chloralose anesthesia the cortex was stimulated through a pair of silver ball electrodes and concentric, stainless-steel electrodes were introduced stereotaxically to stimulate the hypothalamus and other sub-cortical structures. In the first experiments, stimulating electrodes were placed in the preoptic area and anterior hypothalamus and on the surface of the posterior ectosylvian gyrus, the homologue of the first temporal convolution of the primate brain (*2*). A bipolar recording electrode was placed in the lateral nucleus of the amygdala near its juncture with the corticomedial nuclei [F:12, L5, H-6 according to the atlas of Jasper and Ajmone-Marsan (*3*)]. A typical recording electrode site is shown in the photomicrograph of Fig. 1.

Monopolar and bipolar oscilloscopic recordings were made of responses in the amygdala to cortical and subcortical stimulation. Brief rectangular stimuli with a pulse duration of 1 msec were delivered at an intensity just sufficient to evoke reproducible waveforms at the recording electrode. Cortical stimulation elicited a complex triphasic response consisting of a high voltage (400 to 500 μv) 5- to 10-msec sharp potential with a latency of approximately 10 msec, followed by a slow wave of 40 to 70 msec duration appearing about 50 msec after stimulation; occasionally, tertiary waves were observed. Amygdaloid responses to preoptic and anterior hypothalamic stimulation consisted of a broad, 50 to 100 μv, slow wave commencing 20 to 25 msec after stimulation and, occasionally, included a high voltage short-duration component manifest at the crest of the slow wave. Representative traces are shown in Figs. 2 and 3.

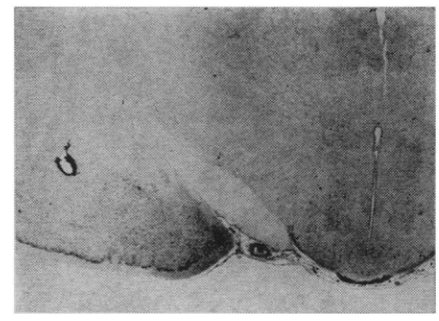

Fig. 1. Photomicrograph of a histological section through the amygdala showing a characteristic recording site represented by a small circular lesion within the substance of the amygdala (Nissl stain).

When sequential stimuli were delivered to both ectosylvian cortex and hypothalamus, the response to the second, or test stimulus, was regularly abolished or attentuated for periods up to 150 msec (Fig. 2). Abolition or attenuation of the response invariably resulted, whether the initial or conditioning stimulation was cortical or hypothalamic. The influence of the conditioning cortical stimulus was somewhat more profound and enduring than that of the test stimulus, but when complete blocking of the high-voltage response to cortical stimulation by prior hypothalamic stimulation occurred, it was no less dramatic.

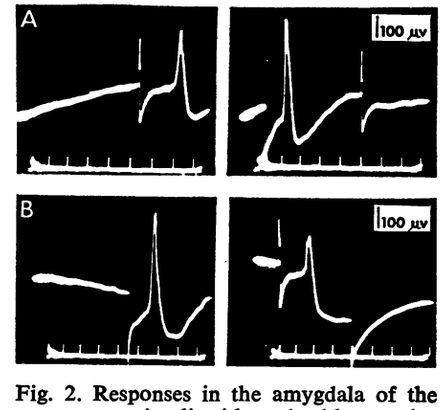

Fig. 2. Responses in the amygdala of the cat to test stimuli with and without prior conditioning stimulation. *A*, Stimulation of the anterior hypothalamus alone and preceded by stimulation of the posterior ectosylvian gyrus with an interval of 50 msec. *B*, Stimulation of the ectosylvian gyrus alone and preceded by stimulation of the anterior hypothalamus with an interval of 40 msec. Bipolar recordings, time scale, 10 msec; calibration, 100 μv.