Table 1. Amino acids in the defatted endosperms of normal and five mutant stocks (expressed as grams per 100 g of protein).

Amino acid	*W64A+*	*W64Ao₂*	*o₁*	*fl₁*	*fl₂**	*fl₂*†	*h*
Lysine	1.6	3.7	1.7	1.8	3.2	3.4	1.8
Tryptophan‡	0.3	0.7	0.6	0.6	0.6	0.9	0.5
Histidine	2.9	3.2	2.3	3.2	2.0	2.4	2.7
Arginine	3.4	5.2	3.3	3.7	4.6	4.3	3.8
Aspartic acid	7.0	10.8	6.1	4.9	7.4	10.9	7.0
Glutamic acid	26.0	19.8	21.5	20.2	17.5	20.6	23.7
Threonine	3.5	3.7	3.3	3.1	3.0	3.6	3.6
Serine	5.6	4.8	4.9	4.7	4.2	5.3	5.7
Proline	8.6	8.6	9.4	11.9	7.6	10.0	10.4
Glycine	3.0	4.7	2.9	2.8	3.0	3.7	3.4
Alanine	10.1	7.2	8.2	7.7	7.3	8.6	9.4
Valine	5.4	5.3	4.7	4.4	4.8	5.6	5.0
Cystine	1.8	[0.9]§	2.2		2.0	1.6	1.5
Methionine	2.0	1.8	2.2	2.5	3.0	3.4	2.8
Isoleucine	4.5	3.9	4.2	3.8	3.8	4.2	4.3
Leucine	18.8	11.6	15.4	15.1	12.7	13.9	16.8
Tyrosine	5.3	3.9	5.0	4.9	4.3	4.7	5.6
Phenylalanine	6.5	4.9	5.7	5.3	4.8	5.4	6.2
			Percent protein				
	12.7	11.1	10.4	10.8	13.6	13.6	10.8

* Grown 1958, analyzed 1964. † Grown 1964, analyzed 1965. ‡ Analyzed by the Spies and Chambers method O (*7*) by J. M. Concon. § Other analyses of *opaque-2* stocks have given cystine values equal to or greater than normal.

dried seeds produced by self-pollination. The endosperms were separated from the embryos and pericarps, ground in a burr mill to a particle size of 0.025 cm, and extracted with hexane (b.p. 65° to 67°C) in a Soxhlet apparatus for 36 hours. The protein content (nitrogen × 6.25) was measured by micro-Kjeldahl assays. Ground, defatted endosperm was hydrolyzed with a 4000-fold excess of 6*N* HCl by refluxing at 110°C for 24 hours with norleucine as an internal standard. Portions (1 ml) of hydrolyzed protein (0.5 mg of protein per milliliter) were then placed on the long and short columns of a Spinco automatic amino acid analyzer.

The amino acid concentrations for a normal (nonmutant) inbred stock and the five mutants are shown in Table 1. The normal inbred was W64A. The *opaque-2* (o_2) stock was a mutant that occurred in the W64A stock and presumably differs from W64A only at the *opaque-2* locus. The analyses for these two stocks give a valid comparison of the changes in amino acid patterns attributable to the *opaque-2* gene. We have analyzed three *opaque-2* mutants from independent mutational events in diverse backgrounds. The differences from normal noted here—increased lysine, arginine, aspartic acid, and glycine together with decreased glutamic acid, alanine, leucine, tyrosine, and phenylalanine—are typical of all *opaque-2* stocks. No such rigorous comparison is possible for the other four mutants, but their amino acid concentrations, relative to those of W64A normal offer reasonable indications of the effect of the mutant gene in question.

Apart from *opaque-2*, only *floury-2* has a major effect on amino acid pattern. For *floury-2*, two analyses of the same line are given. One sample was grown in 1958 and analyzed in 1964, the other was grown in 1964 and analyzed in 1965. For the 1965 analysis, the total recovery was higher (111.7 percent) than the 1964 analysis (95.1 percent), and hence the concentrations of most amino acids are higher. It is apparent that the concentration of lysine is approximately twice that of normal, *opaque-1*, *floury-1* or *soft-starch* and nearly as high as *opaque-2*. The protein content of this *floury-2* line is higher than that of the *opaque-2* line. Thus 100 g of *floury-2* endosperm contains 0.45 g of lysine (from the mean value of the two analyses) compared with 0.41 g for the *opaque-2* line. Further, *floury-2* has a higher methionine concentration (3 to 3.4 g per 100 g of protein) than any line (normal or mutant) tested heretofore. This mutant may be valuable nutritionally in diets where corn is supplemented with kidney beans or soy beans which are low in this amino acid (*6*).

The enhanced lysine content of *opaque-2* stocks results in part from a higher lysine content of the zein (*1*). Preliminary investigations indicate that the lysine content of *floury-2* zein (0.3 g per 100 g zein) is only one-third of that in *opaque-2* zein. In addition, starch-gel electrophoresis of *floury-2* zein gives a pattern different from that of normal and of *opaque-2* zein (*4*). Apparently the biochemical basis of elevated lysine content in *floury-2* is different from that in *opaque-2*, and this suggests the possibility that, in the double mutant stocks now being derived, there may be higher amounts of lysine than in either mutant alone.

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16 August 1965

Biosynthesis of Histones and Acidic Nuclear Proteins under Different Conditions of Growth

Abstract. *The incorporation of uniformly labeled* L-*lysine-C*^{14} *into the normal and regenerating rat liver, into Novikoff hepatoma histones, and into acidic nuclear proteins was studied. In rat liver, different histone fractions incorporate labeled lysine to a different extent. Such differences become less obvious in regenerating liver, and they are even less so in Novikoff hepatoma. In the hepatoma cells the ratio of the biosynthesized acidic nuclear proteins to histones was altered.*

Since the DNA in mammalian chromosomes appears to be associated with histones, Stedman and Stedman (*1*) suggested that histones may act as gene regulators or suppressors. The possibility that histones may act as gene

suppressors focused attention to the studies of histone heterogeneity and species specificity. Such specificity is one of the essential conditions of the gene regulatory function of histones, according to the Stedmans' theory. Tissue specificity of histones was reported by Mauritzen and Stedman (*2*) who described differences in amino acid composition of the arginine-rich β-histones derived from several tissues of ox and domestic fowl. These results were, however, not confirmed when the three main fractions of histones (F1, F2, and F3) from various tissues were analyzed (*3*). Studies performed on the moderately lysine-rich histone fraction F2*b*, purified to a considerable degree of homogeneity, failed to reveal any significant differences in the amino acid composition, in starch-gel electrophoretic mobilities, the number and identity of C- and NH_2-terminal amino acids, C-terminal amino acid sequences, two-dimensional electrophoretic and chromatographic trypsin-digested peptide patterns, or in the composition of the selected trypsin-digested peptides of the F2*b* histones from different tissues of rat, calf, and chicken (*4*).

The controversy concerning tissue and species specificity of mammalian histones indicates that differences in the primary structure of corresponding histone fractions from various tissues are rather small. Possibly tissues differ more in the actual quantities of the histone fractions than in their qualitative composition. Such quantitative differences may be reflected in the rates of the biosynthesis of histone fractions in various tissues.

To investigate this possibility, the incorporation of L-lysine-C^{14}, uniformly labeled, into the four main histone fractions (F1, very rich in lysine; F2*b*, moderately rich in lysine; F2*a* and F3, rich in arginine) was studied in normal and regenerating rat liver and in Novikoff hepatoma. All experimental animals were male albino rats (200 to 250 g).

Rats were injected with the L-lysine-C^{14} intraperitoneallly (20 μc/kg). Subtotal hepatectomy was performed under ether anesthesia (*5*), and the livers were allowed to regenerate for 36 hours. Novikoff hepatoma was transplanted intraperitoneally; 6 days after the transplantation, the animals were injected with labeled lysine.

Nuclei were prepared by homogenization of the tissues in 0.25*M* sucrose containing 2m*M* $CaCl_2$ and 2 m*M* $MgCl_2$, and by density-gradient centrifugation (*3*). Nuclei from Novikoff hepatoma cells were prepared as described (*6*). The nuclei were then washed with 0.14*M* saline containing 0.01*M* trisodium citrate, with 0.1*M* tris buffer (*p*H 7.6), and with 95 percent ethanol. Arginine-rich histones were extracted with a mixture of absolute ethanol and 1.25*N* HCl (4:1 by volume) (*7*). The lysine-rich histones were obtained by extracting the ethanol-HCl residue with 0.2*N* HCl. Both the arginine-rich and lysine-rich histones were further fractionated by gel filtration on Sephadex G-75 into the fractions F1, F2*a*, F2*b*, and F3 (*8*). The nuclear acidic proteins were obtained by extraction of the acid-insoluble residue with 0.1*N* NaOH; the extract was dialyzed against distilled water and lyophilized. The specific activities of the histone fractions were determined by dissolving the dry proteins in water, pipetting 1 mg equivalent to paper discs (Whatman 3 MM), and determining the activity of dried discs in a scintillation (Tricarb) spectrometer. Samples of the acidic nuclear proteins were extracted twice with 5 percent trichloroacetic acid at 90°C for 10 minutes to remove nucleic acids, dried with acetone and ether, weighed, and hydrolyzed in 6*N* HCl at 110°C for 22 hours. The hydrolyzate was then evaporated to dryness at reduced pressure, and the residue was dissolved in distilled water. Portions (1 mg) were pipetted to paper discs (Whatman 3 MM), and their activity was determined as described for the histone fractions. The specific activities of all samples were corrected for lysine content as determined by amino acid analysis of the samples hydrolyzed in 6*N* HCl; for 25 percent of lysine in F1, 15 percent of lysine in F2*b*, 11 percent of lysine in F2*a*, 10 percent of lysine in F3, and 7 percent of lysine in the acidic nuclear protein fraction.

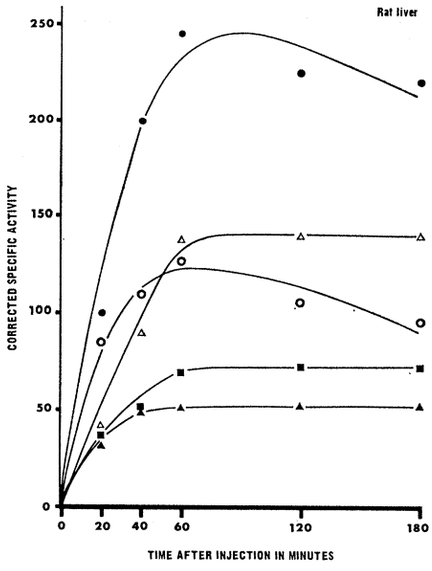

Fig. 1. The incorporation of uniformly labeled L-lysine-C^{14} into nuclear acidic proteins of normal rat liver (●) and histones (△ : F1, ▲ : F2*a*, ■ : F2*b*, and ○ : F3). All specific activities are expressed as counts per minute per milligram corrected for the lysine content of the samples.

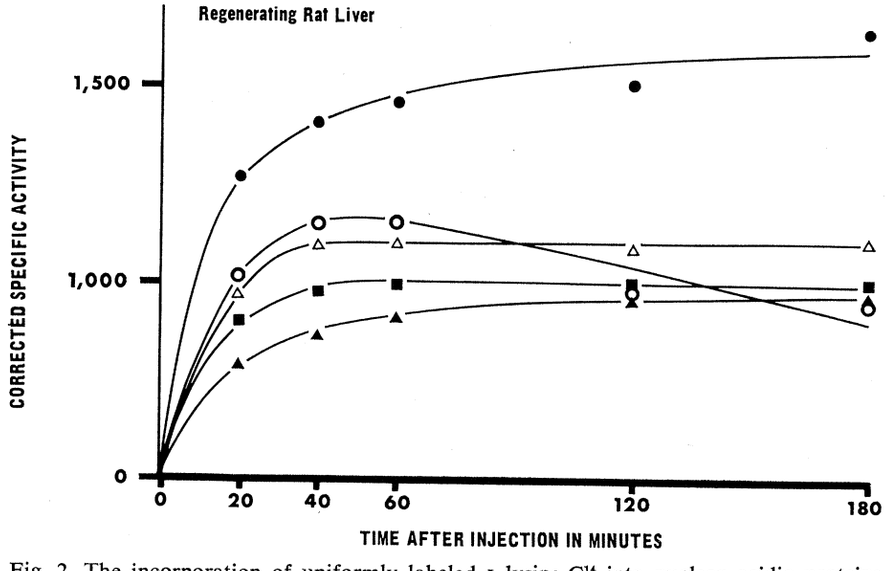

Fig. 2. The incorporation of uniformly labeled L-lysine-C^{14} into nuclear acidic proteins and histones of regenerating rat liver. The symbols are as indicated in Fig. 1.

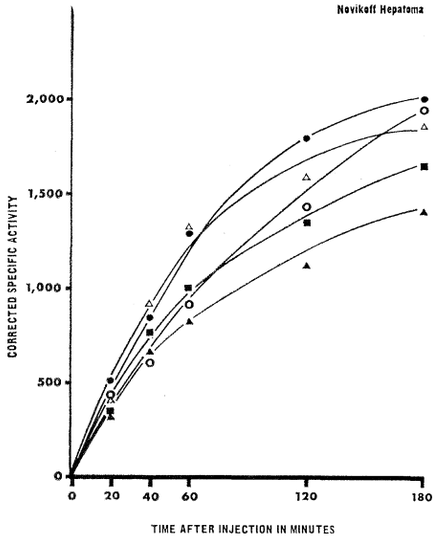

Fig. 3. The incorporation of uniformly labeled L-lysine-C^{14} into histones and nuclear acidic proteins in Novikoff hepatoma cells. The symbols are as indicated in Fig. 1.

In normal rat liver each of the four histone fractions is different in its incorporation of the L-lysine-C^{14}. The arginine-rich fraction 3 and the very lysine-rich fraction 1 incorporated the label most rapidly. The rapid increase in the activity of the fraction 3 histones followed by a decline within approximately 60 minutes after administration of the isotope suggests a high turnover of this fraction. The acidic nuclear proteins are more highly labeled than any of the histone fractions. The incorporation kinetics suggests that histones are biosynthesized more rapidly than the acidic nuclear proteins in liver.

In the regenerating-liver fractions, the incorporation of the isotope was more rapid with an approximately fivefold increase in the specific activity of the histone over that in rat liver. Fraction 3 and fraction 1 are again more active than the fractions 2*a* and 2*b*; however, the differences are much smaller. As in normal rat liver, a rise of activity of fraction 3 is followed by a slow decline, beginning about 1 hour after the administration of L-lysine-C^{14}. In regenerating rat liver as in normal liver, the label appeared faster in histones than in the acidic nuclear proteins. However, the initial differences were much smaller.

In Novikoff hepatoma cells, the incorporation of isotope in the histone fractions was very high (Fig. 3), about 12-fold higher than the values obtained for rat liver, and there is much less difference between fractions in the incorporation of the isotope. In contrast to normal and regenerating liver, there is little difference in the specific activities of the acidic nuclear proteins and of the histones in Novikoff hepatoma.

These data confirm reports that the biosynthesis of basic nuclear proteins increases with increasing rate of cell division (*9*). The different rates of incorporation of L-lysine-C^{14} into various histone fractions of rat liver indicate that the fractions are biosynthesized at different speeds. Dedifferentiation, such as in the first 48 hours of liver regeneration or in hepatoma, substantially lessened the differences in the incorporation of labeled lysine into the four main histone fractions. Rapidly dividing cells seem to have an increased demand for all the histone fractions. The balance between the biosynthesis of the acidic nuclear proteins and the histones becomes upset in favor of the histones. In Novikoff hepatoma, the histones were synthesized at about the same rate as the acidic nuclear proteins. The difference in the biosynthesis of the four main histone fractions, especially in rat liver, support the proposal that different quantities of histones and their structural variations may both be essential to the regulation of genetic activity.

The high rate of biosynthesis of the acidic nuclear proteins in well-differentiated tissues, such as rat liver, suggest that these proteins are closely related to the cellular functions and may be more important in the regulation of nuclear processes than the histones. According to Caspersson (*10*) the most marked change which occurs during prophase is a decrease of the amount of protein in the cell nucleus. The protein which remains is rich in arginine (*11*) and represents most of the basic proteins of the cell (*12*). Our data are compatible with such observations and indicate more specifically that these changes involve mainly the acidic nuclear proteins and all the histone fractions.

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23 September 1965

Parkinsonism: Electromyographic Studies of Monosynaptic Reflex

Abstract. *Electromyographic studies of the monosynaptic reflex in 70 Parkinsonian patients and 12 normal subjects show four types of abnormal facilitation and recovery curves in the Parkinsonian group, types that correspond to variations in the clinical syndrome. Cryosurgical lesions in the ventro-lateral and ventro-postero-lateral nuclei of the thalamus restore essentially normal curves.*

In searching for explanations of the basic neurophysiological events that lead to development of the various signs and symptoms in different neurological disorders, many investigators have concentrated on the alterations observed in the functioning of the most distal elements in the nervous system: namely the motor neuron and the muscle spindle (*1–3*) .

Apart from intrinsic disorders that may affect its function, the motor neuron is constantly exposed to influences from the higher centers that act upon its excitability, either increasing or decreasing its responsiveness or giving special character to the rapidity of its recovery following stimulation (*3, 4*). This superior control can be better studied during two critical periods of physiological activity: one, the brief period