spectra of three of the chemical constituents of muscle have been studied. These constituents were the protein myosin, the carbohydrate glycogen, and the nucleotide adenosine triphosphate. The protein spectrum was not easy to obtain from the undenatured material. Indeed, it is not certain that the spectrum shown in Fig. 2 (curve *G*) represents the absorption in the undenatured state, although the spectrum of dried films is not the same. The myosin was prepared from rabbit muscle according to the method described by Szent-Györgyi (*9*), and the spectrum bears a striking resemblance to the spectrum of frog muscle, although correspondence is surely not complete. More studies of protein extracts and the muscle used for extraction are indicated.

The spectrum of glycogen was very interesting in that an aqueous solution of the material showed absorption almost identical with that of turtle ventricular tissue (*cf.* curves *C* and *D*, Fig. 2). It was concluded, however, that the correspondence between the two spectra was only coincidental, on the basis that a concentration of about 4% glycogen in water is required to duplicate the contour of the muscle tissue spectrum. This is at least four times the maximum normally found in the tissue.

The studies on adenosine triphosphate gave discouraging results in view of the great interest in the physiological significance of this material. Aqueous solutions of ATP in concentrations up to 1% gave completely negative results spectroscopically. In fact, no success was encountered in making water solutions concentrated enough to show absorption bands in the region under consideration, although the solid material absorbs rather strongly. The implication of this result is, of course, that none of the other substances present in small amounts (less than 1%) in muscle can be studied by this technique unless their molar extinction coefficients are considerably larger than that of the ATP. If this is true, the usefulness of the technique will be greatly limited since all but the protein components of muscle will not be detected. Indications are, however, that the protein fraction of the living tissue can be studied through its infrared spectrum. This opens up a large field of study, even though enzyme systems and many metabolic processes are below the threshold of detection.

The results of this preliminary investigation may be stated as follows:

1. It is feasible to measure a useful absorption spectrum of living muscle cells in the infrared region of the spectrum. There appears to be no reason why other living cells will not also yield useful spectra.

2. The useful region of the spectrum of the living tissue is limited to that between 6.5 and 10 μ by the large ratio of water to protoplasm.

3. The spectrum of muscle from 6.5 to 10 μ wavelength shows sharp and strong bands, not all of which occur with regularity in the tissues studied. The absorption band at 9.7 μ is particularly interesting in comparative studies because it appears so strongly in some cells and not at all in others that are physiologically similar.

4. The spectrum of the muscle cell seems to be closely duplicated by that of the protein myosin, although it is certain that other as yet unidentified substances are contributing strongly to the absorption. The substances found in muscle in concentrations less than 1% probably do not contribute to the spectrum, ruling out many biochemical reactions as not being detectable by this technique. The proteins of muscle, however, seem to lend themselves to this method of study.

References

1. CASPERSSON, T. *Skand. Arch. Physiol.*, **73**, Suppl. 8 (1936).
2. COMMONER, B. *Ann. Missouri Botan. Garden*, **35**, 239 (1948).
3. POLLISTER, A. W., and RIS, H. *Cold Spring Harbor Symposia Quant. Biol.*, **12**, 147 (1947).
4. LECOMTE, J., DUBUISSON, M., and MONNIER, A. M. *Arch. intern. physiol.*, **52**, 408 (1942).
5. BLOUT, E. R., and MELLORS, R. C. *Science*, **110**, 137 (1949).
6. BARER, R., COLE, A. R. H., and THOMPSON, H. W. *Nature*, **163**, 198 (1949).
7. HILL, A. V. *Proc. Roy. Soc. (London)*, **B137**, 40 (1950).
8. WOOD, D. L. *Rev. Sci. Instruments*, **21**, 764 (1950).
9. SZENT-GYORGYI, A. *Chemistry of Muscular Contraction.* New York: Academic Press (1947).

Amino Acid Constituents of Prochromosomes Isolated from Blood Cells of Various Animals[1]

G. Yasuzumi and G. Miyao

Faculty of Medicine,
Osaka University, Osaka, Japan

The threadlike bodies isolated from metabolic nuclei apparently resemble in size, appearance, and microstructure the chromosome in anaphase, for which reason they were given the name prochromosomes[2] (*1*). The prochromosomes isolated from blood cells of various animals have been studied with the help of an electron microscope. Each prochromosome is composed of a pair of chromonemata free of DNA and chromatin granules in which DNA are stored (*1, 2*). The chemical constituents of prochromosomes isolated from human leucocyte and carp erythrocyte nuclei, and of salivary chromosomes of *Drosophila,* have been studied by means of paper chromatography and histochemical methods (*3–6*).

The chemical components of chromosomes are of fundamental importance and interest in genetics and cytology. For this reason paper chromatography is one of the most valuable contributions of the chemist to the study of biology. The present paper is an account of our experiments and conclusions concerning the amino acid content of the prochromosomes isolated from blood cells of various animals, chosen from the standpoint of phylogenesis. Although we believe that the present experiments advance our knowledge of the amino acid composition of the prochromosomes, it is clear that much work remains to be done before the complete chemical composition of the gene can be given.

[1] This work was supported in part by a grant from the Science Research Fund, Ministry of Education.

[2] Since this paper was submitted for publication, we have changed this term to "metabolic chromosomes."

TABLE 1

AMINO ACID CONTENT OF PROCHROMOSOMES ISOLATED FROM BLOOD CELLS IN VARIOUS ANIMALS AND OF SALIVARY CHROMOSOMES OF *Drosophila melanogaster*

Amino acid	Prochromosomes isolated from leucocyte nuclei					Prochromosomes isolated from erythrocyte nuclei					Salivary chromosomes (5)
	Man (5)	Rabbit	Guinea pig	Domestic duck	Toad	Cock	Domestic duck	Tortoise	Toad	Carp (4)	
Glycine	+	+	+	+	+	+	+	+	+	+	+
Alanine	+	+	+	+	+	+	+	+	+	+	+
Valine	+	−	+	+	+	+	+	−	+	+	+
Leucine	+	+	+	+	−	+	+	−	−	+	+
Lysine	+	+	+	+	+	+	+	+	+	+	−
Arginine	+	+	+	+	+	+	+	+	+	+	+
Aspartic acid	+	+	+	+	+	+	+	+	+	+	+
Glutamic acid	+	+	+	+	+	+	+	+	+	+	+
Phenylalanine	−	+	−	+	−	+	+	−	−	+	−
Serine	+	+	+	+	+	+	+	+	+	+	−
Threonine	+	−	−	+	−	+	+	+	−	+	−
Proline	−	−	−	−	−	−	−	−	−	−	+
Taurine (?)	+	+	+	+	+	+	+	+	+	+	−

The prochromosomes were isolated from the leucocyte nuclei of rabbit, guinea pig, domestic duck, and toad, and also from the erythrocyte nuclei of cock, domestic duck, tortoise, and toad. The nucleus membranes of blood cells of guinea pig, rabbit, tortoise, and toad were easily destroyed by means of a Waring mixer, but the membranes of cock and domestic duck were so hard that we had to resort to the mechanical action of supersonic waves and the Waring mixer, which were operated alternately to destroy the nuclei completely. The whole procedure was carried out in a cold room at about 1° C. The qualitative methods of paper chromatography and the procedure for isolating prochromosomes were the same as those already described in previous papers (*4, 5*).

In the prochromosomes dissolved in distilled water and not hydrolyzed in NaOH or HCl, no trace of amino acid has been found. Also, no tryptophane has been demonstrated in the specimens hydrolyzed with saturated barium hydroxide or 6 *N* NaOH. A complete list of the amino acids found in the present and previous experiments (*4, 5*) is contained in Table 1, which shows that practically the same amino acids are found in prochromosomes isolated from erythrocyte nuclei of both cock and domestic duck, but the same amino acids are not always found in rabbit and guinea pig. It appears, from evidence presented in this paper, that no qualitative difference is revealed in amino acid composition of the prochromosomes between the nuclei of leucocytes and erythrocytes in the same species. There are some qualitative differences of amino acid composition in the 11 preparations. But even these differences failed to clarify the phylogenetic differentiation that had been expected.

In this experiment glycine, alanine, lysine, arginine, aspartic acid, glutamic acid, serine, and taurine (?) have always been identified in all the prochromosomes isolated from the erythrocyte and leucocyte nuclei. One spot reacting to purple color with ninhydrin appears to correspond to taurine, which has been identified by R_F. However, so far as we are aware, taurine has never before been detected as a component of proteins. Whether the spot is taurine or a degradation product of other materials remains to be determined. It was considered to be "taurine" in our previous papers (*4, 5*) and in the present study. Proline has been demonstrated in the salivary chromosomes of *Drosophila* but has never been demonstrated in the prochromosomes isolated from blood cells.

In subsequent experiments we have investigated whether DNA has interfered with the appearance of tryptophane on paper chromatograms. A solution of 0.1% tryptophane in saturated barium hydroxide or 6 *N* NaOH, containing 0.1–1.0% DNA, was heated at 110° C. After 15 hr one drop of the solution was placed on the paper (Toyo, No. 2). In this experiment only the butanol-acetic acid solvent was used for identifying tryptophane, which was calculated to be 5 or 10 γ. It has been confirmed that the presence of DNA does not interfere with the reaction of tryptophane on the paper chromatogram.

The greatest uncertainty in this work lies not in the result of the analytical procedures but rather in the difficulty of getting a sufficiently pure specimen. As already shown by electron microscopy (*1, 2*), it was comparatively easy to isolate the pure prochromosomes from the metabolic nuclei of blood cells.

If there is to be a phylogenetic chemo-differentiation within the prochromosome protein components, it is probably quantitative rather than qualitative. Moreover, it apparently does not occur at the level of the amino acids, but on a higher plane of molecular organization.

References

1. YASUZUMI, G., *et al. Chromosoma* (in press).
2. YASUZUMI, G. *Ibid.*
3. KIRBY, B. *Proc. Natl. Acad. Sci.*, **34**, 561 (1948).
4. YASUZUMI, G., and MIYAO, G. *Exptl. Cell Research*, **1**, 501 (1950).
5. *Ibid.* (In press.)
6. YASUZUMI, G., *et al. Cytologia*, **15**, 173 (1950).