ently the dehydrogenase action of xanthine oxidase is not interfered with by Antabuse. In view of these facts, in order for Antabuse to have an effect in the whole organism one might expect some time to elapse in order to allow all the dehydrogenase function to be reduced, before any true inhibition may be observed. This was well borne out by our studies, since we found that a period of 3 days of Antabuse dosing was necessary before the thiopental potentiation became evident. It might be argued that the 3 days were necessary because of the cumulative dosage, but this hardly seems pertinent since the individual doses were massive, the 25 mg/mouse representing about 1,250 mg/kg.

Finally, these data lead to the inference that xanthine oxidase is one enzyme functioning in the metabolism of thiopental to thiopental carboxylic acid. Further work designed to substantiate this inference will be reported later.

It is concluded that Antabuse, possibly through its inhibitory effect on xanthine oxidase, has the ability to prolong markedly the anesthesia induced in the mouse by intravenous thiopental. Pending investigations in higher mammals, it is suggested that thiopental be administered with caution to individuals who are undergoing treatment with Antabuse.

References

1. BRODIE, B. B., *et al. J. Pharmacol. Exptl. Therap.*, **98**, 85 (1950).
2. GIARMAN, N. J., and FLICK, F. H. Unpublished data.
3. HALD, J., ASMUSSEN, E., and LARSEN, V. *Acta Pharmacol. Toxicol.*, **4**, 311 (1948).
4. ERRIKE, J. *Quart. J. Studies Alc.*, **11**, 183 (1950).
5. RICHERT, D. A., VANDERLINDE, R., and WESTERFELD, W. W. *J. Biol. Chem.*, **186**, (1), 261 (1950).

Infrared Microspectrum of Living Muscle Cells[1]

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The absorption spectra of many animal and plant tissues have been reported in the literature. For the ultraviolet, several authors (*1–3*) have reported successful microspectra of tissue cells and parts of cells, both living and fixed. For the infrared the macrospectra of dried tissues (*4, 5*) and the microspectra of individual dried cells (*6*) have been reported. Preliminary spectroscopic measurements on living muscle cells in the wavelength region from 1 to 15 μ were undertaken by the author to establish the feasibility of using infrared spectroscopy as a tool in studies of living tissues. These measurements are still incomplete, but the data so far collected are presented to show that the difficulties recently reported (*7*) in measuring useful microspectra of living tissues are not insurmountable.

[1] Part of this work was done under an AEC predoctoral fellowship in the Biological Sciences at Ohio State University.

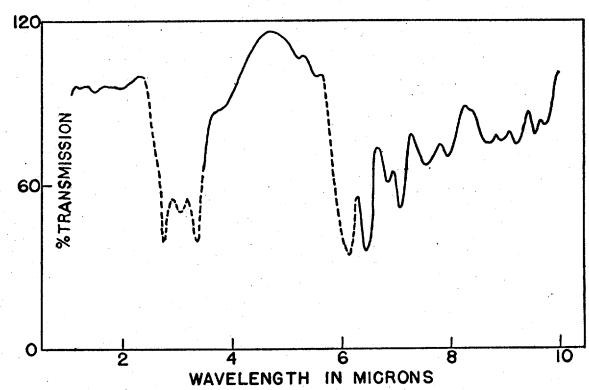
[2] USPHS postdoctoral fellow, National Institutes of Health.

It seemed desirable to use the individual cellular unit of muscle for this work, both because of the difficulty of preparing suitable specimens in any other form, and because in some cases a variation is known to exist in the nature and function of different cells from the same muscle. For successful spectroscopy of such small specimens (20 μ–180 μ diam) in the infrared, it was necessary to use the microspectrometer described elsewhere (*8*), which was originally designed for studies of small fibers.

The specimens were prepared as follows: From the freshly isolated muscle a fiber was teased out under Ringer's solution. The isolated fiber was mounted in Ringer's between transparent plates (fluorite or silver chloride) and placed in the radiation beam of the microspectrometer. The spectrum was then recorded. If the fiber was too small to give a satisfactory spectrum, from 1 to 4 fibers were laid side by side to form a larger absorbing area. The spectra were considered to be representative of the living tissue if the fiber (or fibers) could be made to contract visibly on electrical stimulation after the spectrum had been measured. It was not always possible to measure the whole spectrum before the contractility of the fiber was lost. The interesting region from 6.5 to 10 μ could be examined conveniently in two sections, although it was possible in some cases to get through the whole region without losing contractility.

The principal difficulty in measuring the infrared spectrum of any living tissue is the large ratio of water to protoplasm. It is well known that water is a very strong absorber of infrared radiation. As a result, the absorption bands of the muscle are nearly obliterated by the very intense absorption of the water. The difficulty was resolved in this case by using a spectrometer of maximum sensitivity and measuring the absorption of the cells relative to the absorption of the aqueous suspension medium. The ratio of energy transmitted through the cell to that transmitted through the same thickness of Ringer's solution next to the cell was plotted against wavelength to obtain the spectrum.

The spectrum from 1 to 10 μ for a fiber from the gastrocnemius of a frog is shown in Fig. 1. It was



SPECTRUM OF A MUSCLE CELL FROM THE FROG GASTROCNEMIUS

FIG. 1.

found that beyond 10 μ the absorption of the muscle fiber in the preparations studied was identical with that of an equal thickness of Ringer's solution. In the region from 6.5 to 10 μ there are several strong and sharp absorption bands (to be discussed later). In the region from 5.7 to 6.5 μ the absorption of the muscle is obscured by the strong band of liquid water in that region. At wavelengths shorter than 6 μ the spectrum has not been intensively studied. The very interesting 3-μ region is almost completely obscured by the strong water band at that wavelength, and between 3.5 μ and 6 μ the absorption bands of the preparations used were weak and diffuse. The dotted portions of the curve were drawn to represent the spectrum of the tissue that one would expect on the basis of the dehydrated material. The region from 6.5 to 10 μ seems to be the most useful portion, according to present information.

In this region the eight bands listed in Table 1, with their wavelengths and frequencies, occurred with enough intensity and regularity to be considered real. The bands not tabulated have not been studied extensively as yet.

TABLE 1

Band designation (Fig. 2)	Wavelength (μ)	Frequency (cm^{-1})
a	6.50	1,538
b	6.85	1,460
c	7.10	1,408
d	7.65	1,307
e	8.00	1,250
f	8.5 –8.7	1,175–1,150
g	9.2	1,087
h	9.7	1,031

It was found that, although many of the bands were present in the spectra of all the muscle tissues studied, there were some differences among the various fibers. The bands marked *a–g* in Fig. 2 (curve *F*) occurred in all muscle spectra, as well as that of the extracted protein myosin (curve *G*, Fig. 2). However, the band marked *h* in Fig. 2 (curve *F*), falling at 9.7 μ, was not found in all the spectra, as can be seen by comparing curves *A, B, D, E, F* in Fig. 2. The band was strongest in the frog gastrocnemius fibers, and in turtle ventricular muscle. It was totally absent, or extremely weak, in frog ventricular muscle, frog sternohyoideus muscle, and many others. The occurrence of the band is tabulated in Table 2, with the minus signs indicating the absence of the band, and the number of plus signs indicating roughly the intensity of the band if it did occur.

Every entry in Table 2 represents at least two different spectra, each necessarily of different fibers, and each usually from a different muscle. A total of 77 spectra is represented. No correlation has been observed between the presence or absence of this band and any physiological or structural differences in the muscles.

Other differences appearing in the spectra, such as

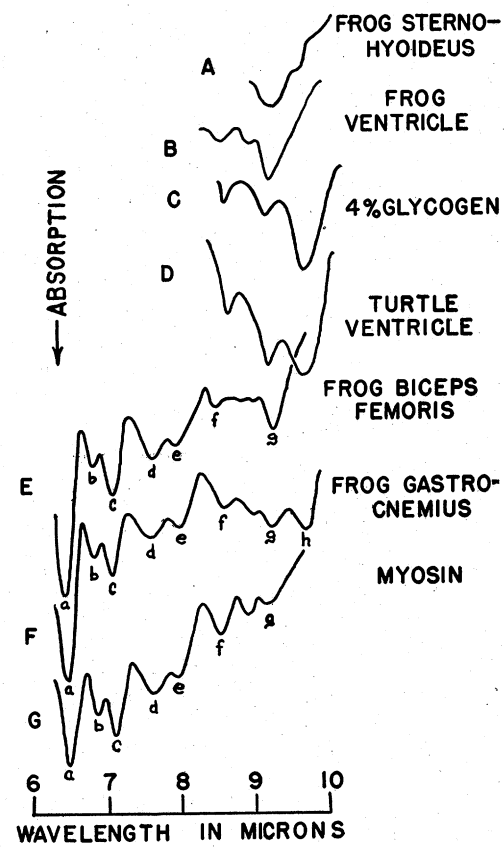


FIG. 2.

the presence of the small band between those marked *f* and *g* in Fig. 2 (curve *F*) have not been studied well enough at present to be discussed here. Further work is in progress.

In addition to the muscle spectra themselves, the

TABLE 2

Animal	Muscle	Band at 9.7 μ
Frog	Tibialis anticus	++
"	Sartorius	++
"	Gastrocnemius	++
"	Sternohyoideus	–
"	Hyoglossus	–
"	Ventricle	–
"	Biceps femoris	–
Turtle	Ventricle	+++
"	Auricle	+++
"	Rectus abdominis	++
"	Pectoralis	+
"	Neck, retractor	+
"	Trapezius	+ (weak)
"	Extensor digitorum	–
"	Mixed red and white	±
Cockroach	Wing	–
"	Leg	–
Rabbit	White	–
"	Red	–

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]

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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."