Residue 38, being susceptible to $T_1$ ribonuclease, is presumably a G (or I) derivative. Its occurrence adjacent to the anticodon is an exception to the proposal that tRNA's whose code words begin with U have a modified A at this position (*18*).

Finally, that it was possible to assemble the fragments from $T_1$ and pancreatic ribonuclease digests into the correct sequence by means of regularities that have emerged from known tRNA sequences emphasizes not only the essential correctness of these generalities, but also the extent to which they reflect functional requirements (*19*). The similarities among tRNA sequences suggest not only a common evolutionary origin, but also a severely restricted tolerance to mutation. This limited tolerance implies restraints which must derive from the integration of multiple functions into one molecule, with a resultant well-defined tertiary structure: hence, the close relation between tRNA functions and native tertiary structure (*3*), and the marked topographical similarity among tRNA's (*20*).

STEVEN KOWALSKI, TETSUO YAMANE
JACQUES R. FRESCO

*Department of Biochemical Sciences, Frick Chemical Laboratory, Princeton University, Princeton, New Jersey*, and *Medical Research Council Laboratory for Molecular Biology, Cambridge, England*

### References and Notes

1. Abbreviations used: tRNA, transfer ribonucleic acid; $tRNA^{Leu}_3$, "denaturable" leucine acceptor tRNA; A, adenosine; C, cytidine; G, guanosine; U, uridine; D, dihydrouridine; I, inosine; $\psi$, pseudouridine; T, ribothymidine; mC, 5-methylcytidine; 2′omG, 2′,*O*-methylguanosine; p, on the left (of G) indicates a 5′-phosphate on that residue; OH on the right as in $A_{OH}$ indicates the absence of a 3′-phosphate, that is, a free 3′-hydroxyl group; $tRNA^{Met}$, $tRNA^{Tyr}$, $tRNA^{Ile}$, methionine, tyrosine, and isoleucine accepting tRNA, respectively.
2. T. Lindahl, A. Adams, J. R. Fresco, *Proc. Nat. Acad. Sci. U.S.* **55**, 941 (1966).
3. J. R. Fresco, A. Adams, R. Ascione, D. Henley, T. Lindahl, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 527 (1966).
4. T. Lindahl, A. Adams, M. Geroch, J. R. Fresco, *Proc. Nat. Acad. Sci. U.S.* **57**, 178 (1967).
5. A. Adams, T. Lindahl, J. R. Fresco, *ibid.*, p. 1684.
6. T. Lindahl, A. Adams, J. R. Fresco, *J. Biol. Chem.* **242**, 3129 (1967).
7. S. Kowalski and J. R. Fresco, *Science*, this issue.
8. F. Sanger, G. G. Brownlee, B. G. Barrell, *J. Mol. Biol.* **13**, 373 (1965); G. G. Brownlee and F. Sanger, *ibid.* **23**, 337 (1967); ———, B. G. Barrell, *ibid.* **34**, 379 (1968).
9. G. G. Brownlee and F. Sanger, *Eur. J. Biochem.* **11**, 395 (1969).
10. See review by M. Staehelin, *Experientia* **27**, 1 (1971).
11. Some of these features are summarized in G. R. Philipps, *Nature* **223**, 374 (1969); M. Levitt, *ibid.* **224**, 759 (1969).
12. T. Lindahl, D. Henley, J. R. Fresco, *J. Amer. Chem. Soc.* **87**, 4961 (1965).
13. S. K. Dube, K. A. Marcker, A. Yudelevich, *Fed. Eur. Biochem. Soc. Lett.* **9**, 168 (1970).
14. H. M. Goodman, J. A. Abelson, A. Landy, S. Brenner, J. D. Smith, *Nature* **217**, 1019 (1968).
15. S. Cory, K. A. Marcker, S. K. Dube, B. F. C. Clark, *ibid.* **220**, 1039 (1968).
16. L. H. Schulman and R. W. Chambers, *Proc. Nat. Acad. Sci. U.S.* **61**, 308 (1968).
17. S. Takemura, M. Murakami, M. Miyazaki, *J. Biochem. Tokyo* **65**, 489 (1969).
18. D. J. Armstrong, W. J. Burrows, F. Skoog, K. L. Roy, D. Söll, *Proc. Nat. Acad. Sci. U.S.* **63**, 834 (1969); F. Skoog and D. J. Armstrong, *Annu. Rev. Plant Physiol.* **21**, 359 (1970).
19. This does not mean that it will be possible to similarly deduce the sequences of all tRNA's. Indeed, we do not wish to suggest that such deduction replace unambiguous structure determination.
20. J. R. Fresco, R. D. Blake, R. Langridge, *Nature* **220**, 1285 (1968); R. D. Blake, J. R. Fresco, R. Langridge, *ibid.* **225**, 32 (1970).
21. H. G. Zachau, D. Dütting, H. Feldman, *Hoppe-Seylers Z. Physiol. Chem.* **347**, 212 (1968).
22. M. Staehelin, H. Rogg, B. C. Baguley, T. Ginsberg, W. Wehrli, *Nature* **219**, 1363 (1968).
23. J. Gangloff, G. Keith, J. P. Ebel, G. Dirheimer, *Nature, New Biol.* **230**, 125 (1971).
24. We thank B. G. Barrell for helpful advice, and Dr. F. Sanger for the hospitality of his laboratory for much of this work. This research was supported in part by NIH grant GM 07654, NSF grant GB 18865, and the American Heart Association. S.K. was supported by predoctoral traineeships from PHS grant GM 962. T.Y. is a staff member of Bell Telephone Laboratories, Murray Hill, New Jersey. J.R.F. was supported in part by a fellowship from the John Simon Guggenheim Memorial Foundation.

1 February 1971

## Striated Muscle Fibers: Facilitation of Contraction at Short Lengths by Caffeine

Abstract. *One of the factors evidently responsible for decreasing the force of muscle contraction with shortening is inactivation of the myofibrils in the core of a muscle fiber. Caffeine antagonizes this inactivation and, correspondingly, changes the length-force relationship at short muscle lengths.*

The relationship between the length of a frog striated muscle fiber and the force it can actively produce shows a plateau of maximum force at striation spacings of 2.0 to 2.2 $\mu$m (*1*). As the length is increased above 2.2 $\mu$m, force drops linearly toward zero at 3.65 $\mu$m (Fig. 1). As explained by the sliding filament theory (*2*), this results from a decrease in overlap between thick and thin filaments. On the other side of the plateau, force decreases to zero at 1.3 $\mu$m. One of the reasons for this decrease is that the degree of activation evidently becomes less as a muscle shortens, for during shortening initiated by membrane depolarization the myofibrils in the core of a fiber become wavy, showing that their activation has been inhibited (*3*). To determine the degree to which this factor influences the length-force relationship at short striation spacings, we observed the effect of caffeine, which is known to facilitate activation (*4*), on contractions of frog skeletal muscle.

Single muscle fibers, isolated from the semitendinosus muscle of *Rana temporaria*, were mounted horizontally in a trough filled with Ringer solution sitting on the stage of an ordinary light microscope. Their tendons were fastened to steel hooks, one of which was stationary, the other attached to an RCA 5734 force transducer. The latter was mounted on a rack and pinion that was used to vary the fiber length. For contractions below slack length (about 2.0 $\mu$m) the fiber was allowed to hang in a parabola between the hooks. On stimulation it took up the slack until it had reached the predetermined striation spacing before producing force isometrically. Cine micrographs (Fig. 2) were taken while simultaneously force was being measured. Striation spacings were, in general, measured on enlargements made from the cine film. Occasionally, however, striations could not be unequivocally resolved at lengths below about 1.3 $\mu$m, and then we calculated the final length

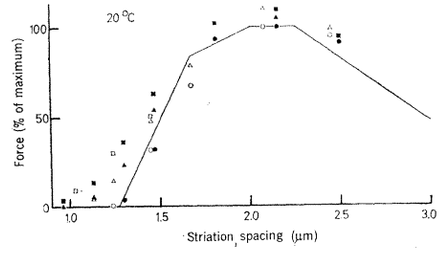


Fig. 1. The influence of caffeine upon the length-force relationship of isolated muscle fibers. Force, on the ordinate, is the maximum amount recorded during contractions elicited by tetanic stimulation (77 hertz) at a particular striation spacing. The open and filled symbols refer to two single fiber preparations. Circles, triangles, and squares are the responses in 0, 2, and 3 m*M* caffeine Ringer solution, respectively. The solid line is a summary of the data taken from Gordon *et al.* (*1*), for comparison with our control responses.

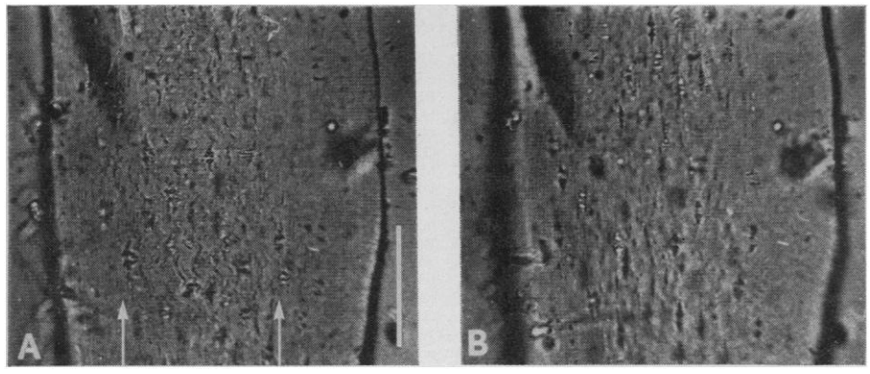


Fig. 2. Selected cine micrographs of an isolated fiber that has shortened to 1.3 $\mu$m during tetanic stimulation. Temperature was 20°C. Calibration mark represents 50 $\mu$m. Exposure time 2 msec. (A) In normal Ringer solution. Wavy myofibrils are visible between the arrows delineating the part that has become inactivated. The force was 0.04 times that at slack length. (B) In Ringer solution containing 3 m*M* caffeine. All the myofibrils appear to be straight. The force was 0.23 times that at slack length.

from the distance between the ends of the fiber. The striation spacings determined by this method differed from the values measured on the photographs by less than 0.05 $\mu$m. Stimulation was transverse by a pair of bright platinum plate electrodes, each tetanus coming at 1-minute intervals. The general techniques have been described previously (*3*, *5*).

Preliminary studies showed that contractures with shortening induced by 5 to 10 m*M* caffeine produced considerably more force than tetani at the same short length. The results, however, were regarded as unreliable, because these contractures had a time course much slower than that of a tetanus, caused the striation spacing along a fiber to be very irregular, and usually ended with at least part of the fiber irreversibly shortened. These difficulties did not arise when we compared cine micrographs of tetani in normal Ringer solution to those in Ringer solution containing caffeine in concentrations that did not themselves produce shortening. At such concentrations, caffeine is believed to lower the threshold for contractions initiated by action potentials, thus making a given action potential more effective in activating contraction, particularly as regards the peak magnitude of force development in a twitch (*4*). We found that the maximum force developed during a tetanus can also be increased by the addition of caffeine. Figure 1 shows the length-force relationships at 20°C for two single fibers. Caffeine, being somewhat more effective at 3 m*M* than at 2 m*M* concentration, increased the force at the plateau of the relationship by 5 to 10 percent. This supports the idea that muscle can reversibly develop force significantly greater than that usually thought to reflect the maximum load-bearing capacity (*6*). But the action of caffeine was particularly obvious at the shorter lengths: at 1.3 $\mu$m, where, in agreement with the findings of Gordon, Huxley, and Julian (*1*), our fibers had ceased to produce force in the control solution, they still generated up to 35 percent of maximum force during tetani in 3 m*M* caffeine. Moreover, they were able to shorten reversibly and produce force at striation spacings as short as 1 $\mu$m. Correspondingly, the wavy myofibrils that appeared in the core of a shortened fiber in the control solution, remained straight during shortening to the same length in the presence of caffeine (Fig. 2). Caffeine, therefore, can sustain the activation of the central myofibrils that usually become inactivated during shortening induced by membrane depolarization. This action is correlated with an increase in the ability to develop force at short lengths and can be explained by the idea that caffeine improves the effectiveness of membrane depolarization in producing activation.

We thus support the conclusion drawn from our previous findings (*3*), namely, that an important factor that contributes to determining the length-force relationship as a muscle shortens is a decreasing degree of activation.

REINHARDT RÜDEL*
STUART R. TAYLOR†
*Department of Physiology, University College London, London, W.C. 1, England,* and *Department of Pharmacology, State University of New York, Downstate Medical Center, Brooklyn 11203*

**References and Notes**

1. A. M. Gordon, A. F. Huxley, F. J. Julian, *J. Physiol. (London)* **184**, 170 (1966).
2. A. F. Huxley, *Progr. Biophys. Biophys. Chem.* **7**, 255 (1957); H. E. Huxley, in *The Cell*, J. Brachet and A. E. Mirsky, Eds. (Academic Press, New York, 1960), vol. 4, p. 365.
3. S. R. Taylor and R. Rüdel, *Science* **167**, 882 (1970).
4. S. R. Taylor, H. Preiser, A. Sandow, *J. Gen. Physiol.* **54**, 352 (1969).
5. R. Rüdel and S. R. Taylor, *J. Physiol. (London)* **205**, 499 (1969).
6. A. L. Hodgkin and P. Horowicz, *ibid.* **153**, 386 (1960); M. Brust, *Amer. J. Physiol.* **208**, 425 (1965); K. Fujii and T. Sakai, *Jikei Med. J.* **16**, 75 (1969).
7. We thank A. F. Huxley, in whose laboratory most of this work was done, for his suggestions and hospitality, and we thank him and D. R. Wilkie for reading and criticizing the manuscript. We thank E. B. Ridgway for many thought-provoking discussions and D. Kenny for technical assistance. This study was supported by the Deutsche Forschungsgemeinschaft and by PHS grant NS 08973-01 from the NIND&S.
* Present address: II. Physiologisches Institut der Universität, 69 Heidelberg, Bergheimer Strasse 147, West Germany.
† Present address: Department of Pharmacology, State University of New York, Downstate Medical Center, Brooklyn, New York 11203.

25 September 1970; revised 19 November 1970 ■

## EEG Responses in Regularly Menstruating Women and in Amenorrheic Women Treated with Ovarian Hormones

Abstract. *Electroencephalographic driving reponses to photic stimulation vary with the menstrual cycle and with manipulations of ovarian hormones thought to control the menstrual cycle. Estrogens reduce driving responses to photic stimulation, and estrogen plus progesterone enhance these responses. The electroencephalographic changes may reflect the effects of gonadal steroid hormones upon central adrenergic processes.*

The gonadal steroid hormones, estrogen and progesterone, are suspected of playing significant roles in brain functions (*1*, *2*). We report here that driving responses to photic stimulation varied with the menstrual cycles of normal females (study 1) and could be manipulated by administrations of estrogen and progesterone to amenorrheic women (study 2). Fewer electroencephalographic (EEG) driving responses occurred in the preovula-