Table I summarizes the results. No consistent change in the volume or acidity of the different phases of the gastric secretion is noted as a result of histidine injection. It might be argued that these observations were made after a single dose of histidine and therefore might not detect the effect of the drug on repeated administration. The patient in Case 14 received this medicine on 6 consecutive days and the study carried out on the 3rd and 6th days, respectively, also yielded negative results. Incidentally, this patient derived no apparent benefit from the therapy, while a standard Sippy regimen which soon followed brought on the usual expected remission.

It may thus be concluded that under the conditions of these observations histidine does not seem to exert any influence on the gastric acidity or the rate of gastric secretion.

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Contraction of Muscle and Denaturation of Myosin.

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It is recognized that the protein myosin takes part directly in the contraction of muscle, and there is even reason to believe that myosin itself is the contractile substance of muscle.[1, 2] If so significant a rôle in contraction is ascribed to myosin, it is important to know how myosin changes during contraction. Experiments concerning the effect of heat on both muscle and myosin show that, under some conditions at least, shortening of muscle may be made possible by the denaturation of myosin. In this note I shall briefly describe these experiments.

A thermal stimulus can cause either a reversible or an irreversible shortening of muscle. If the sartorius muscle of a frog is gradually warmed, its length remains unchanged until a temperature of about 37° is reached, when the muscle suddenly shortens. On cooling, the muscle recovers its original length. If the muscle is heated to higher temperatures a further shortening is observed, beginning at 39° and reaching an end-point at about 45°C. This time the muscle does not relax on cooling. The 2 responses of muscle to heat were first

[1] Weber, H. H., *Ergeb. d. Physiol.*, 1934, **36**, 109.

[2] Muralt, A. J., *Ergeb. d. Physiol.*, 1935, **37**, 406.

sharply distinguished from each other by Jensen,[3] who referred to the reversible shortening as *thermal contraction* and to the irreversible shortening as *thermal rigor.*

In experiments on the effect of heat on myosin isolated from frog muscle I find that heat denatures myosin in 2 distinct steps, one of which can be identified with thermal contraction and the other with thermal rigor. In these experiments myosin was in a medium bearing some resemblance to its environment in the muscle fiber—in isotonic KCl with a small amount of phosphate adjusting the pH to 6.9. Under these conditions very little protein is in solution and myosin has the appearance of a transparent semi-fluid gel. When such a preparation is heated to 37° no very striking change in appearance is observed, although on more careful comparison with an unheated sample it is seen that the gel is darker and less transparent. On examining the solubility of myosin heated at 37° it is found that a pronounced change has indeed taken place. If unheated myosin is added to an excess of cold 1.2 M KCl brought to pH 8.0 with K_2HPO_4 practically all the protein dissolves, but much remains insoluble if the myosin is heated to 37° and the quantity of insoluble protein is subsequently estimated by adding 1.2 M KCl in the cold. The temperature coefficient of this change is of the order of a thousand reckoned for a change of 10°, so that at 39° a few minutes' heating suffices to render nearly all the myosin insoluble. Under these conditions there is only a slight change in the SH groups of myosin. As myosin is heated to temperatures over 39° a striking change appears. Beginning at about 41° (the exact point depending upon the rate of heating) the gel draws together into a number of firm opaque clumps. Changes in the SH groups of myosin can now be detected, and by estimation[4] of SH groups the temperature coefficient of this process has been found to be much lower than that of the first process. Both the change in solubility observed at 37° and the change in SH groups observed at higher temperatures may be regarded as steps in the denaturation of myosin because in other proteins, notably egg albumin, it has been found that loss of solubility, appearance of SH groups and an extraordinarily high temperature coefficient are the most striking characteristics of the process of heat denaturation. In egg albumin loss of solubility and change in SH groups appear to occur at the same time.[5] I shall refer to the 2 steps in the denaturation of myosin as primary (at 37-38°) and secondary denaturation (40-45°).

[3] Jensen, P., *Arch. ges. Physiol.*, 1914, **160**, 333.

[4] Mirsky, A. E., and Anson, M. L., *J. Gen. Physiol.*, 1935, **18**, 307.

[5] Mirsky, A. E., and Anson, M. L., *J. Gen. Physiol.*, 1936, **19**, 427.

Primary denaturation of myosin can be identified with thermal contraction and secondary denaturation with thermal rigor. At the temperature of thermal contraction it is found that myosin in muscle becomes insoluble with only slight changes in its SH groups, and in thermal rigor the protein SH groups of muscle change as they do in secondary denaturation. With respect to opacity, hydration and other qualities, significant comparisons can be made between contraction of muscle and the denaturation of myosin. Experiments on rabbit muscle are of interest because in this case heat produces changes similar to those observed in the frog but at different temperatures. In the skeletal muscles of the rabbit the first effect of heat on contraction was stated by Claude Bernard[6] to appear at 45° (in contrast to 37° for the frog). I find that the primary denaturation of isolated rabbit myosin takes place at about 45°. The significance of the difference between the myosins of frog and rabbit is emphasized by comparing the myogens of frog and rabbit muscle. Myogen is an albumin-like protein in muscle, no change in which is detectable as a consequence of contraction. The myogens of frog and rabbit are denatured at the same temperature, 54°, and in this protein loss of solubility and change in SH groups occur in one step.

In contraction of muscle due to other stimuli than heat there are reasons to suppose that primary denaturation of myosin occurs; the extent of shortening in thermal contraction is of the same order as that produced by electrical stimulation;[3] and in those types of contraction in which the changes in myosin have been investigated there is observed a loss of solubility without change in SH groups.[7]

Summary. Myosin isolated from frog muscle is denatured by heat in two distinct steps: the first, occurring at 37°, is characterized by a high temperature coefficient (about a thousand for 10°) and no change in SH groups; the second, occurring between 40°-45°, has a much lower temperature coefficient and a marked change in SH groups. In the thermal stimulation of muscle two different kinds of contraction occur, a reversible contraction at 37° and an irreversible contraction at higher temperatures. The reversible contraction can be correlated with the first step in denaturation of myosin, and the irreversible contraction can be correlated with the second step in denaturation of myosin.

[6] Bernard, Claude, referred to on page 194 in Kühne, W., *Myologische Untersuchungen*, Leipzig, 1860.

[7] Mirsky, A. E., *J. Gen. Physiol.*, 1936, **19**, 559.