

these vesicles and therefore appear in the soluble protein fraction of MH susceptibles. This possibility could be addressed by Blanck *et al.*, or others, by comparing preparations derived from the supernatant of a low-speed centrifugation (ca. 20,000 $\times g$) with that of a much higher speed centrifugation (ca. 100,000 $\times g$). In both cases the presence or absence of microsomes should be confirmed. There still remains the possibility of serum contamination, which is unavoidable in preparing the samples.

We do not recommend the method of diagnosis proposed by Blanck *et al.*^{1,2} until the nature of these proteins is understood more clearly.

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In reply:—We appreciate the efforts of Fletcher and Rosenberg to clarify the putative electrophoretic differences between MH and normal muscle. Although we never stated that electrophoresis should be used as a diagnostic criterion for MH, we do agree with them that the extra protein bands are probably hemoglobin. We have determined this in three ways. MH and control muscle proteins and a standard hemoglobin sample on the SDS gels were transferred to nitrocellulose paper. The transferred patterns were then stained for hemoglobin with benzidine. The benzidine reaction stains heme proteins in general, therefore, positive staining with benzidine could relate to any other heme protein as well as hemoglobin. The low molecular weight proteins from the MH sample stained positively for hemoglobin; no staining was evident in the electrophoretic sample from the control patient. The hemoglobin sample transferred to the nitrocellulose paper as expected stained positively for hemoglobin. We also took an aliquot of the supernatant from an MH and a normal control patient and directly added benzidine to them and found that the MH sample was markedly positive for hemoglobin while the control sample only showed a trace amount of staining. Gel Isoelectric® focusing of hemoglobin and the MH muscle samples demonstrated a yellowish band in unstained gels at exactly the same pI for both hemoglobin and MH samples. This further substantiates that the extra MH proteins are probably only hemoglobin

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contaminants. Finally, vastus lateralis samples from several more patients who have undergone orthopedic surgery have also demonstrated the extra protein found in their gel electrophoretic patterns that we had originally found only in the MH patients.

The question arises as to why the original control samples did not contain the hemoglobin contaminants that were found in the MH patients muscle? The most obvious explanation is that half of the six control patients underwent knee surgery and had a tourniquet applied to their thigh during the time when the muscle specimen was obtained. This resulted in less bleeding, and therefore less hemoglobin contamination was evident in our biopsy specimen. The other patients fortuitously had less traumatic surgery compared with our MH patients, resulting in less hemoglobin contamination.

In conclusion, electrophoresis of MH muscle samples cannot be used at this time to distinguish MH from normal muscle. Future investigations, however, may yet demonstrate differences in protein composition of MH compared to normal muscles.

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