7. A. Marshak-Rothstein, J. D. Benedetto, R. L. Kirsch, M. Gefter, *J. Immunol.* **125**, 1987 (1980).
8. E. Lamoyi, P. Estess, J. D. Capra, A. Nisonoff, *J. Exp. Med.* **124a**, 2834 (1980).
9. A. Marshak-Rothstein, M. Siekevitz, M. N. Margolies, M. Mudgett-Hunter, M. L. Gefter, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1120 (1980); S. S. Alkan, R. Knecht, D. G. Braun, *Hoppe-Seylers Z. Physiol. Chem.* **361**, 191 (1980); M. Margolies, A. Marshak-Rothstein, M. L. Gefter, *Mol. Immunol.* **18**, 1065 (1981); M. Siegelman, C. A. Slaughter, L. J. McCumber, P. Estess, J. D. Capra, in *Immunoglobulin Idiotypes*, C. Janeway, E. Sercarz, H. Wigzell, Eds. (Academic Press, New York, 1981), pp. 135–158; C. A. Slaughter and J. D. Capra, unpublished observations.
10. C. A. Slaughter, M. Siegelman, P. Estess, I. Barasoain, A. Nisonoff, J. D. Capra, in *Developmental Immunology: Clinical Problems and Aging*, E. L. Cooper and M. A. B. Brazier, Eds. (Academic Press, New York, in press).
11. P. Early, H. Huang, M. Davis, K. Calame, L. Hood, *Cell* **19**, 981 (1980).
12. G. Matthyssens and T. H. Rabbitts, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6561 (1980).
13. H. Sakano, R. Maki, Y. Kurosawa, W. Roeder, S. Tonegawa, *Nature (London)* **286**, 676 (1980).
14. Abbreviations for the amino acid residues are: Ala, alanine; Arg, arginine; Asp, aspartic acid; Asn, asparagine; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine.
15. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
16. D. L. Bentley and T. H. Rabbitts, *Cell* **24**, 613 (1981).
17. T. H. Rabbitts, G. Matthyssens, P. H. Hamlyn, *Nature (London)* **284**, 238 (1980).
18. C. A. Slaughter and J. D. Capra, in preparation.
18a. P. Estess, personal communication.
19. R. S. Jack, T. Imanishi-Kari, K. Rajwesky, *Eur. J. Immunol.* **8**, 559 (1977).
20. M. Reth, T. Imanishi-Kari, K. Rajewsky, *ibid.* **9**, 1004 (1979).
21. A. L. M. Bothwell, M. Paskind, M. Reth, T. Imanishi-Kari, K. Rajewsky, D. Baltimore, *Cell* **24**, 625 (1981).
22. R. Lieberman, M. Potter, E. B. Mushinski, W. Humphrey, S. Rudikoff, *J. Exp. Med.* **13**, 983 (1974); J. L. Claflin, *Eur. J. Immunol.* **6**, 669 (1976); P. J. Gearhart, N. D. Johnson, R. Douglas, L. Hood, *Nature (London)* **291**, 29 (1981).
23. S. Crews, J. Griffin, H. Huang, K. Calame, L. Hood, *Cell* **25**, 59 (1981).
24. E. Selsing and U. Storb, *ibid.*, p. 47; M. Pech, J. Hochtl, H. Schnell, H. G. Zachan, *Nature (London)* **291**, 668 (1981).
25. L. J. McCumber, M. Qadeer, J. D. Capra, *Methods in Peptide and Protein Sequence Analysis*, C. Birr, Ed. (Elsevier/North-Holland, Amsterdam, 1980), pp. 165–172.
26. T. H. Rabbitts, J. M. Jarvis, C. Milstein, *Cell* **6**, 5 (1975).
27. M. P. Wickens, G. N. Buell, R. T. Schimke, *J. Biol. Chem.* **253**, 2483 (1978).
28. D. W. Strafford and D. Bieber, *Biochim. Biophys. Acta* **378**, 18 (1975).
29. T. E. Shenk, C. Rhodes, P. W. J. Rigby, P. Berg, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 989 (1975).
30. N. M. Gough, E. A. Webb, S. Cory, J. M. Adams, *Biochemistry* **19**, 2702 (1980).
31. M. W. McDonnell, M. N. Simon, F. W. Studier, *J. Mol. Biol.* **110**, 119 (1977).
32. R. Roychouhury, E. Jay, R. Wu, *Nucleic Acids Res.* **3**, 105 (1976).
33. D. Hanahan, personal communication.
34. M. Grunstein and D. S. Hogness, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3961 (1975).
35. W. Dunnick, T. H. Rabbitts, C. Milstein, *Nucleic Acids Res.* **8**, 1475 (1980).
36. A. M. Maxam and W. Gilbert, *Methods Enzymol.* **65**, 499 (1980).
37. N. Blin and D. W. Stafford, *Nucleic Acids Res.* **3**, 2303 (1976).
38. D. T. Denhardt, *Biochem. Biophys. Res. Commun.* **23**, 641 (1966).
39. P. J. W. Rigby, M. Dieckmann, C. Rhodes, P. Berg, *J. Mol. Biol.* **113**, 237 (1977).

40. Supported in part by NIH grants AI12127 and AI18016 and NSF grant PCM 76-22411. J.S. was supported by Damon Runyon–Walter Winchell Cancer Fund grant DRG-298F and NIH grant AI-06020. We thank Ana Garcia-Spain, Sandy Graham, Martha Danhof, and Alan Forster for technical assistance and Kathy Able for assistance in typing the manuscript.

* Address requests for reprints to J.D.C.

22 January 1982

Decreased Insulin Receptors but Normal Glucose Metabolism in Duchenne Muscular Dystrophy

Abstract. *Compared to matched controls, 17 patients with Duchenne muscular dystrophy showed decreased insulin binding to monocytes due to decreased receptor concentration. These patients showed no signs of altered glucose metabolism and retrospective analysis of the clinical records of a further 56 such patients revealed no modification in carbohydrate metabolism. These data suggest that reduced insulin receptor number does not produce overt modifications of glucose metabolism in Duchenne muscular dystrophy.*

The concentration or the affinity of the insulin receptor changes in response to physiological, pharmacological, or pathological variations in glucose metabolism. However, it is not known whether modification of the insulin receptor per se provokes variation in glucose metabolism. In this report we describe a clinical situation in which modification of the insulin receptor status does not appear to produce overt disturbances of glucose metabolism.

The monocytes of an apparently normal 5-year-old boy showed fewer insulin receptors compared to the monocytes of other matched boys. No reason could be found to explain this finding. About 2 years later, however, the boy was found to have muscular dystrophy of the Duchenne type (DMD). DMD is a systemic lesion that is inherited as an autosomal dominant trait (*1*) and is characterized by marked membrane defects (*2*); a modification of the insulin receptor is thus to be expected. However, DMD is also characterized by normal carbohydrate metabolism (*1*), which would not lead one to expect an alteration of the insulin receptor; to our knowledge, in fact, defects in the insulin receptor in the absence of overt changes of carbohydrate metabolism have not been reported. This led us to study the insulin receptor in males with DMD.

The studies were carried out on 17 males with DMD (aged 8 to 23 years) from the Istituto S. Stefano in Porto Potenza Picena (Italy). All patients had a history of pseudohypertrophy plus progressive proximal weakness, marked increase in creatine phosphokinase (CPK), and electromyography typical of DMD. Diagnosis had been made 2 to 15 years previously. The control group consisted of 17 males (aged 8 to 25 years) whose body weights were 91 to 112 percent of the ideal (*3*) and whose family histories indicated no diabetes or obesity. Insulin binding to monocytes in DMD patients was lower than in controls ($P < .001$) (Fig. 1); conversely, no differences were found in plasma glucose or serum insulin values (data not shown). Six handicapped subjects with neurogenic atrophy (non-DMD) from the same hospital (receiving the same diet and leading the same physical life as the DMD patients) showed normal insulin binding to monocytes, thus confirming that the results were not due to the life-style of the DMD patients (data not shown). The wide range of data in the DMD patients (Fig. 1) was not due to abnormal insulin bind-

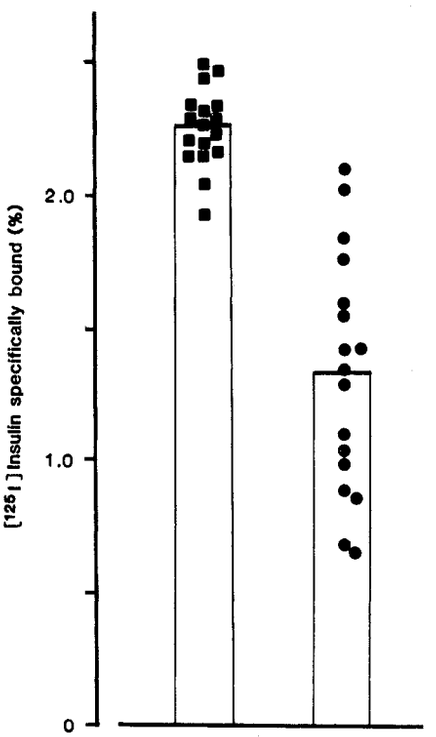

Fig. 1. Insulin binding to monocytes (4×10^6 per milliliter) from normal subjects (■) and DMD patients (●). All subjects were on an unrestricted diet and were not taking any drug known to affect carbohydrate or insulin metabolism. Blood (70 ml) was collected at 0800 hours after overnight fasting and samples were centrifuged on a Ficoll-Angiografin gradient. Mononuclear cells were isolated, resuspended in tris-HCl buffer (*p*H 7.8) to a final concentration of 3×10^7 per milliliter, and subsequently incubated for 100 minutes at 15°C with [^{125}I]insulin (0.6 ng/ml; CEA-IRE Sorin, Italy; 180 to 200 μCi/μg) in the absence or presence of increasing amounts of porcine insulin (Organon, Holland) (*9*). Student's *t*-test for unpaired data was used in the statistical analysis.

0036-8075/82/0416-0311$01.00/0

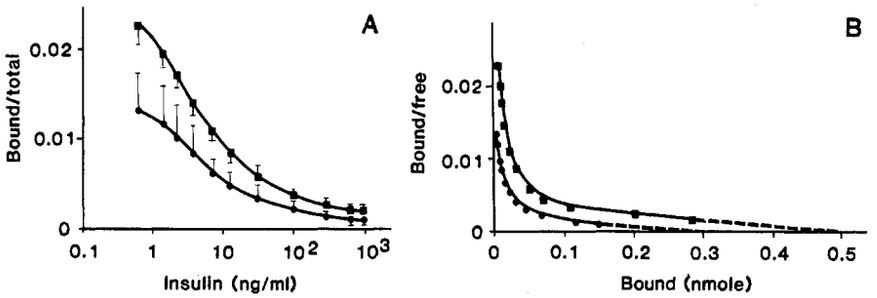

Fig. 2. (A) The inhibiting effect of porcine insulin on [^{125}I]insulin binding to monocytes (4×10^6 per milliliter) from control subjects (■) and DMD patients (●). Values represent means ± standard error. (B) The corresponding Scatchard plot. At the lowest ratio of bound to free radioactivity, the data become much less precise because of the low counts; this makes it difficult to identify the exact intercept on the x-axis of the curve (an extrapolation) representing R_0 (total receptor number) and consequently the K_e value (average affinity at empty sites). Because of this, and because the presence of one or more binding sites remains to be elucidated, analyses of changes in K_e and R_0 or the so-called high- and low-affinity binding sites have not been performed, but rather Scatchard plots were used in order to reach qualitative conclusions on receptor concentration or receptor affinity variations.

ing variation from day to day, since five of them, studied on several occasions, showed minor changes which are within the range of changes in normal man (*4*).

Competition-inhibition studies showed that binding was lower in DMD patients than in controls at any given insulin concentration (Fig. 2A), thus suggesting that the insulin binding variation was due to a change in receptor concentration. This was confirmed by Scatchard analysis of the data (Fig. 2B).

Many factors (*4*, *5*) may induce variations in insulin binding. In the DMD patients we found no relation between insulin binding and serum insulin, duration of the disease, steroid (sexual or adrenal) or CPK levels; furthermore, we found no circulating antibodies, such as insulin or receptor antibodies, to explain the reduced receptor concentration (*6*) (data not shown). Thus the cause of the impairment remains obscure. One possibility is that decreased receptor number is genetically induced. This hypothesis will be evaluated by studying fibroblasts for DMD patients (*7*).

With the exception of one member, all the family members of three patients showed normal receptor concentration and receptor affinity on various occasions. The exception was a 22-year-old man with no sign of DMD (physical examination; normal electromyography and CPK) or modified carbohydrate metabolism (oral glucose and insulin tolerance tests as well as body weight were within the normal range). This subject continuously showed reduced insulin binding to monocytes because of reduced receptor number (about one-half). He had two normal sisters and one brother with DMD. One explanation for this finding might be that the insulin receptor is a marker (genetic?) of membrane defects even in cases showing no overt clinical evidence of DMD.

It was not possible to assess the insulin sensitivity in DMD patients because of their marked vascular fragility and reluctance to participate in the study. However, plasma glucose and serum insulin after an oral glucose tolerance test (100 g) performed in 14 out of the 17 DMD patients and in 15 matched normal subjects showed similar behavior (data not shown).

To obtain further data on the metabolic effects of DMD, we studied retrospectively the clinical records of a further 56 DMD patients. This number of patients is large relative to the incidence of the disease: 2.8 per 100,000 (*1*). Most of the records included all the clinical data up to the time of death of the patients. In only one case was a slight (~ 130 mg per 100 ml of serum) and brief (3 months) hyperglycemia noted; this condition disappeared spontaneously without therapy or change of diet. In none of the records was there any mention of therapies or diets used in the managements of alterations in carbohydrate metabolism.

Thus DMD is characterized by low insulin receptor concentration despite the absence of impaired glucose tolerance or evidence of modified carbohydrate metabolism. To our knowledge this is the first report of decreased insulin receptor number not being accompanied by overt changes of glucose metabolism or clear signs of insulin resistance. What is the possible significance of these data? Insulin action is due to the interaction of the hormone with its receptors and the subsequent generation of a signal (or signals) activating effectors responsible for the biological effects of insulin (postreceptor events); thus, normal insulin action depends on the normal function of receptors and postreceptor events. In DMD, however, it appears that the decreased receptor number does not modify glucose metabolism. Insulin exerts its maximum biological effects by occupying a small percentage of receptors (*5*, *8*); the remaining receptors are, presumably, unoccupied and all potentially are fully functional; thus one possible explanation is that the insulin receptor number in DMD is still sufficient to maintain glucose homeostasis and, therefore, the lack of evident changes in receptor affinity (Fig. 2) might be the key to explain the metabolic situation in DMD. Disturbances in glucose metabolism might appear in the presence of modifications in receptor affinity or a marked reduction of the receptor number or both. This would mean that in many cases the receptor defect does not induce, but is induced by, the metabolic alteration.

Another, but less likely, possibility is that DMD is characterized by a complex alteration involving enhancement of postreceptor events able to perfectly counteract the decreased receptor concentration. These data show that decreased receptor concentration does not necessarily produce overt modifications in glucose metabolism and thus indicate that we should look more carefully into the problem of the real effect of receptor variations (concentration and affinity) in glucose metabolism; furthermore, they suggest that considerable caution should be exercised in ascribing modifications of glucose metabolism to insulin receptor defects.

ROBERTO DE PIRRO*
2ª Clinica Medica, Policlinico Umberto 1°, Università di Roma, 00161 Rome, Italy
RENATO LAURO
Cattedra di Medicina Costituzionale ed Endocrinologia, Università Cattolica del Sacro Cuore, Rome, Italy
IVANO TESTA
GINOFABRIZIO FERRETTI
CARLO DE MARTINIS
Clinica Medica, Università di Ancona, Ancona, Italy
RENZO DELLANTONIO
Istituto Policattedra delle Malattie del Sistema Nervoso, Università di Ancona

References and Notes

1. J. N. Walton, *Disorders of Voluntary Muscle* (Churchill Livingstone, London, 1974), pp. 561–599.
2. E. Bonilla, D. L. Schotland, Y. Wakayama, *Ann. Neurol.* **4**, 117 (1978); J. L. Howland and S. L. Iyer, *Science* **198**, 309 (1977); R. I. Sha'afi, S. B. Rodan, R. L. Hintz, S. M. Fernandez, G. A. Rodan, *Nature (London)* **254**, 526 (1975); G. A. Rao, K. Siler, E. C. Larkin, *Science* **200**, 1416 (1978); A. D. Roses and S. H. Appel, *Nature (London)* **250**, 245 (1974).
3. Geigy Table (J. R. Geigy S.A., Basle, ed. 7, 1977).
4. R. De Pirro, A. Fusco, A. Bertoli, A. V. Greco,

R. Lauro, *J. Clin. Endocrinol. Metab.* **47**, 1387 (1978); A. Bertoli, R. De Pirro, A. Fusco, A. V. Greco, R. Magnatta, R. Lauro, *ibid.* **50**, 246 (1980).
5. D. Andreani, R. De Pirro, R. Lauro, J. Roth, J. M. Olefsky, *Current Views on Insulin Receptors* (Academic Press, London, 1982).
6. J. S. Flier, C. R. Kahn, J. Roth, R. S. Bar, *Science* **190**, 63 (1975); R. De Pirro, A. Fusco, L. Spallone, R. Magnatta, R. Lauro, *Diabetologia* **19**, 118 (1980).
7. C. R. Kahn and J. M. Podskalny, *J. Clin. Endocrinol. Metab.* **50**, 1139 (1980); M. J. Prince, P. Tsai, J. M. Olefsky, *Diabetes* **30**, 596 (1981).
8. T. Kono and R. W. Barham, *J. Biol. Chem.* **246**, 6210 (1971); J. Gliemann, S. Gammeltoft, J. Vinten, *ibid.* **250**, 3368 (1975).
9. R. De Pirro, A. Bertoli, A. V. Greco, A. S. Gelli, R. Lauro, *Acta Endocrinol. (Copenhagen)* **90**, 473 (1979).
10. This work was performed with a grant from ISIS (sezione scientifica).
* To whom correspondence should be sent.

29 October 1981; revised 3 February 1982.

Mycoplasma pneumoniae Infection: Role of a Surface Protein in the Attachment Organelle

Abstract. *Attachment of* Mycoplasma pneumoniae *to host cells by means of a specialized terminus initiates infection. Monoclonal antibodies to a surface protein (P1) inhibit this process, and react with a region of the tip covered with peplomer-like particles. Since antibodies against the P1 protein are generated by natural and experimental infection and by immunization, the substance may be an important determinant of protective immunity.*

In earlier studies of the interaction of *Mycoplasma pneumoniae* with host cells in tracheal organ cultures (*1*), we showed that the attachment of viable mycoplasmas to the respiratory epithelium is necessary for the initiation of infection. Biberfeld and Biberfeld (*2*) first reported an internal rod within a narrow, slightly knobbed tip at one end of filaments of *M. pneumoniae*. Collier (*3*) observed that this differentiated terminal structure always occurred in apposition to host cell membranes and suggested that it was important for the attachment of *M. pneumoniae*. This observation was supported by later studies of radioactively labeled mycoplasmas (*4*) and of sputum samples from patients (*5*). Subsequently, we demonstrated that a surface protein component was involved in attachment (*6*). When *M. pneumoniae* was treated with proteases, attachment was inhibited and a major protein band (P1) was diminished on gel electrophoresis (*6*). Reincubation of these trypsin-treated *M. pneumoniae* in growth medium resulted in regeneration of the P1 protein and resumed ability of the organism to attach to respiratory epithelium. However, direct evidence for the P1 protein on the tip structure of *M. pneumoniae* has not been reported.

Recently, we isolated hybridoma cell lines that produce monoclonal antibodies against the surface antigens of *M. pneumoniae* (*7*). By using the protein blot and radioimmunobinding techniques (*8*) we found that one of the hybridoma cell lines, M-218, secretes an immunoglobulin IgG1 (antibody M-218) that is directed specifically against the P1 protein. In this report we demonstrate, by using this monoclonal antibody, that the P1 protein is localized at the attachment tip of *M. pneumoniae*. We also demonstrate by negative staining electron microscopy that a well-demarcated outer layer is restricted to the area of the terminal structure of *M. pneumoniae*.

Mycoplasma pneumoniae strain M-129 (ATCC No. 29342) was originally isolated from a patient with pneumonia (*9*). After several passages in vitro, *M. pneumoniae* was grown either in Hayflick medium supplemented with 20 percent horse serum from which gamma globulins had been removed (*6*) or in SP-4 medium (*10*). The cultures were prepared as described previously (*6*). In some experiments, the monolayers were treated with trypsin (*6*). Electrophoresis was performed in 10 percent sodium dodecyl sulfate (SDS)–polyacrylamide slab gels, and the separated proteins were transferred to nitrocellulose sheets which were then used for immunoradiobinding studies (*8*). The Coomassie blue–

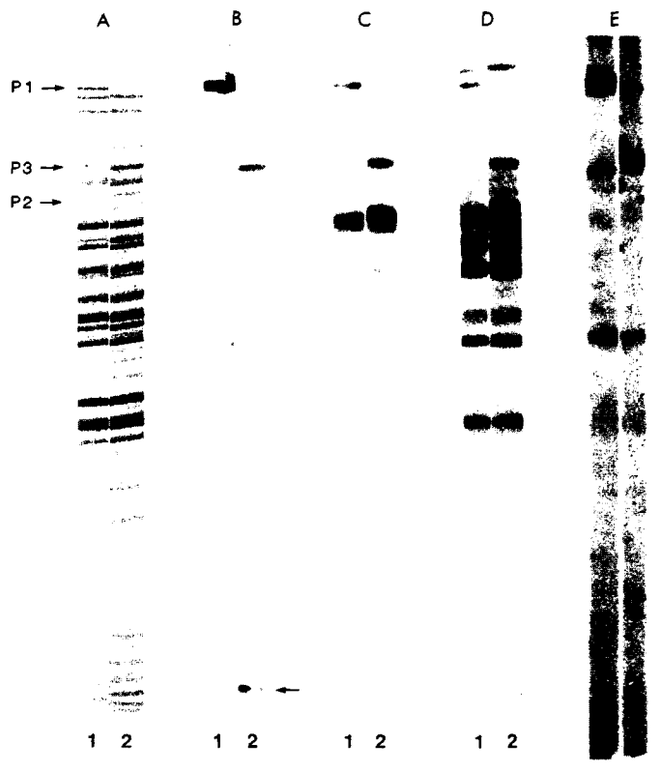

Fig. 1. Immunoradiobinding studies of *M. pneumoniae* proteins. (A) Sodium dodecyl sulfate (SDS)–polyacrylamide gels of 1, untreated and 2, trypsin-treated organisms stained with Coomassie blue. Note the absence of both the P1 (molecular weight 190,000) and P2 (molecular weight, 78,000) protein bands and the appearance of the P3 (molecular weight, 90,000) band in the trypsin-treated preparation. (B) The proteins of *M. pneumoniae* shown in (A) were transferred from SDS-polyacrylamide gel to nitrocellulose sheets, incubated with ascitic fluid containing monoclonal antibodies, and imaged with ^{125}I-labeled rabbit antibody to mouse IgG. Radioautography indicates the specific binding of monoclonal antibody to the P1 protein (B1) and P3 (B2); the latter is a residual polypeptide of P1 since it retains the same antigenic determinant. An additional residual protein band (molecular weight, ~ 12,500) which also can be recognized by the antibody is located at the bottom of the gel (B2, indicated by the arrow). (C, D, E) Same as (B), but incubated with serum from (C) a hamster infected with *M. pneumoniae* by inhalation, (D) a rabbit hyperimmunized by injection of *M. pneumoniae*, and (E) a patient with confirmed mycoplasmal pneumonia, and then "imaged" with ^{125}I-labeled respective species-specific second antibodies. Antibodies specifically against P1 protein are present in all cases, indicating that this substance is a major immunogen of the mycoplasma.

0036-8075/82/0416-0313$01.00/0