Humerus of Robust Australopithecus

McHenry's (*1*) discriminant analysis of the large robust *Australopithecus* humerus, KNM-ER 739, from strata east of Lake Rudolph, Kenya (*2*), demonstrated that it is morphologically and, presumably, functionally distinct from the humeri of extant large hominoids. He concluded that his results could not determine whether this early hominid used its forelimbs for both manipulation and locomotion or just for manipulation. I suggest that his data do contribute a possible solution to this problem.

I have estimated the values for each hominoid taxon on the first discriminant function of McHenry's study from his figure 1 (*1*). Among extant hominoids which use their forelimbs in locomotion, these values are significantly correlated with body weight ($r = -.95$, $P < .01$). The regression equation for body weight (W) in this group is $W = 305 - 25F_1$, where body weight is in pounds and F_1 is the value of the first discriminant function. In a plot of F_1 against W (Fig. 1), *Homo sapiens* lies well away from the other extant hominoids. The body weight of *Homo* estimated from the first discriminant function is less than one-fourth the known body weight for this species; man has a humerus which is smaller than would be expected for a hominoid of his body size. This is probably related to the fact that man's forelimbs are not used in locomotion.

Body weights for the robust *Australopithecus* have been estimated at between 135 and 200 pounds (~ 60 to 90 kg). A body weight of 143 pounds (65 kg) would be assigned to KNM-ER 739 from the regression equation for hominoids which use their forelimbs in locomotion. Therefore, the humerus of robust *Australopithecus* was of a size to be expected if it was used for locomotion, unless the estimates of body weight are grossly incorrect (*3*).

If the forelimbs of *Homo sapiens* are smaller because they are not used in locomotion, the absence of a similar reduction in the forelimbs of robust *Australopithecus* implies that these animals were using their forelimbs in some form of locomotion. The possibility should be considered that robust *Australopithecus* species were facultative rather than habitual bipeds.

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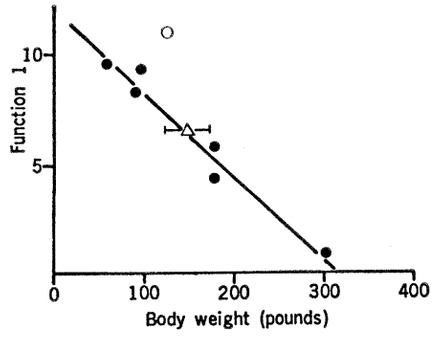

Fig. 1. Plot of McHenry's (*1*) first discriminant function against body weight; (closed circles) extant hominoids which use their forelimbs in locomotion; (open circle) *Homo sapiens*; (triangle) fossil *Australopithecus* humerus KNM-ER 739. The regression line was calculated for hominoids which use their forelimbs during locomotion.

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Kay's point is well taken and he could be entirely correct in suggesting that the robust *Australopithecus* used its forelimbs in some form of locomotion. However, his argument depends on the estimated body weight of the robust *Australopithecus*, which he gives as between 135 and 200 pounds. These estimates are based primarily on the South African robust forms which are smaller than the hyper-robust hominids of East Africa. The individual represented by the KNM-ER 739 humerus might have weighed much more than Kay's prediction of 143 pounds. Certainly, some of the fossil femurs from the East Rudolf site indicate that a very large bodied hominid was present. If the body weight of the KNM-ER 739 individual was 225 to 250 pounds (102 to 113 kg), the humerus would bear the same relationship to Kay's regression line as does *Homo sapiens*.

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Insulin Activity: The Solid Matrix

The topic of discussion between Katzen and Vlahakes (*1*) and Cuatrecasas (*2*) on Cuatrecasas's experiments (*3*) on the biological activity of insulin coupled covalently to agarose is of far-reaching importance. Prior to 1968 there was a large amount of evidence, albeit indirect, indicating that most polypeptide hormones, including insulin, stimulated target cells by interacting with receptors on the cell surface. In 1968 Schimmer *et al.* (*4*) reported that the polypeptide hormone ACTH covalently linked to large cellulose particles stimulated adrenal cells, and this activity was unaccounted for by solubilization of ACTH. In 1969, in a similar but more extensive study, Cuatrecasas reported that insulin covalently linked to agarose (Sepharose) particles was almost as potent as native insulin (*3*). These data have been cited as a major direct experimental support to widely held notions that polypeptide hormones act through surface receptors.

In his experiments Cuatrecasas incubated isolated fat cells with insulin coupled to agarose beads and found that the immobilized insulin was nearly as potent as native insulin. Katzen and Vlahakes agreed with Cuatrecasas that insulin most likely acts at the cell membrane and that insulin coupled to agarose may be biologically active, but they felt that the studies by Cuatrecasas needed clarification. In particular, Katzen and Vlahakes recalculated Cuatrecasas's published data and concluded that in several key experiments there was less than one insulin-agarose bead per incubation flask. This conclusion was based on the fact that Cuatrecasas used insulin-agarose preparations containing 171, 320, and 360 μg of insulin per milliliter of agarose (*3*) and that 1 ml of agarose has about 5×10^5 beads per milliliter (*1*). They stated that one was "faced with a dilemma of explaining how it would be operationally possible to dilute a sus-

pension of insulin-Sepharose equivalent to about 17 microunits (1 microunit = 0.04 ng) of insulin immobilized per bead (calculated from 340 μg of insulin coupled per milliliter) to a range of 2 to 10 microunits of insulin-Sepharose per final volume (*1*).

Cuatrecasas contended that the conclusions of Katzen and Vlahakes were incorrect since "they assumed that the insulin-agarose derivatives used for the biological assays were the identical ones which were used to characterize the chemical linkage form of the derivatives" (*2*). "The chemical studies (enzymic digestions, sulfitolysis, amino acid analyses, and so forth) obviously require much higher concentrations of insulin than do the biological studies" (*2*). Cuatrecasas then states that the preparations used for his studies of biological activity actually contained "20 ng to 1 μg of insulin per milliliter of agarose" (*2*).

We are very surprised by Cuatrecasas's reply for several reasons. Cuatrecasas denoted (*3*, table 1, p. 451), that the preparations containing 171 to 360 μg of insulin per milliliter of agarose "were used for studies of biological and immunological properties." We have been unable to find in Cuatrecasas's publication (*3*) any mention of insulin derivatives containing less than 171 μg of insulin per milliliter of Sepharose, much less of derivatives coupled at nanogram concentrations or descriptions of methods of coupling suitable to these low hormone concentrations (*3*). Further, in order for Cuatrecasas to claim that "effects occur with concentrations of insulin-Sepharose that are nearly as low as those of native insulin" (*3*), he must have been able to determine with accuracy the quantity of insulin that was linked to the agarose. The method of amino acid analysis of the insulin-agarose, described by him for this determination (*3*), is suitable for microgram amounts of coupled insulin, but would be too insensitive for accurate analysis of nanogram amounts of coupled insulin. We also can find no method published by Cuatrecasas for this crucial determination.

We agree that most, if not all, of the actions of insulin do occur as a result of the hormone interacting with its receptor on the cell membrane. Further, we agree that experiments with hormones coupled to a solid matrix can provide important information about their mechanisms of action. However, until important clarifications and corrections are brought forward, the studies reported by Cuatrecasas are exceedingly difficult to interpret as they now stand.

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The report (*1*) describing the biological activity of insulin-agarose derivatives has proved to be important because it has provided a stimulus for extensive, independent studies of insulin receptors. Five years later these studies have progressed to the point where they confirm and overshadow the conclusions which could have been derived at that time from the original data. Furthermore, the original report led us to use derivatives to achieve extensive purification of membrane-solubilized insulin receptors by affinity chromatography (*2*). In addition, it may have been at least a partial stimulus for the use of similar approaches in totally different fields which have, for example, led to important contributions in the areas of lymphocyte mitogenesis (*3*), fractionation of immunocompetent cells (*4*), catecholamine- and histamine-cell interactions (*5*), antiviral effects of interferon (*6*), and acetylcholine receptor purification by biospecific adsorption (*7*).

Butcher *et al.* (*8*) are correct that the original report (*1*) did not contain sufficiently detailed information concerning the analyses used to quantitate some of the derivatives used in the biological studies. After establishing the basic conditions (*p*H and the use of acetylated insulin) which dictate the predominant chemical form of the linkage, a great many derivatives were prepared under these same conditions, but with varying concentrations of insulin so that in certain derivatives very low degrees of substitution were achieved. In the latter, the quantity of insulin incorporated was determined by radioimmunoassay procedures [which were published in 1969 (*9*) and which were apparently overlooked by Butcher *et al.* (*8*)] especially adapted for use with these insulin-agarose derivatives, or by the use of ^{3}H-labeled (*9*) (8 to 14 c/mmole) or ^{125}I-labeled (20 to 500 c/mmole) insulin, methods that can readily and reliably detect microgram and nanogram quantities of matrix substitution. Other criticisms could also be made, especially in retrospect, of the work reported in (*1*). Because of space limitations, the report did not adequately describe that the dilutions of the insulin-agarose derivatives were always made with unsubstituted agarose. Also, the control data showing that the media used in the reported incubations of insulin-agarose with cells did not contain significant amounts of free insulin were not presented, the intrinsic chemical stability of the protein-agarose bond was not described, and the quantitative relation between protein substitution and biological potency was not discussed.

None of these criticisms, however, detract from the validity of the basic findings and conclusions of (*1*), and Butcher *et al.* (*8*) do not disagree with the conclusions. The biological activity of these derivatives has now been amply confirmed in our own and other (*10*) laboratories. Moreover, strong and specific physical interaction of fat cells with insulin beads has recently been directly demonstrated by Katzen and colleagues (*11*), and large, soluble insulin-dextran polymers have

now been shown to be biologically active (*12*). Furthermore, the conclusions are completely consistent with a large body of other information which has since accumulated concerning insulin receptors. It is important to realize, however, that the mechanisms by which insoluble insulin derivatives activate cells are probably very complicated and different from those that normally occur with native insulin (*13*). This area of understanding is constantly expanding and there are still many uncertainties and experimental limitations.

The merits and weaknesses of (*1*) should be judged 5 years later in context of the rapid and extensive developments that have since transpired in the field of hormone receptors in particular and membrane receptors in general. For example, important problems in the area of insulin receptors which currently merit intensive thought and investigation include the isolation and purification of receptor and related membrane structures with the ultimate hope of reconstituting an active system in vitro, determination of the role of membrane fluidity in receptor function, and elucidation of the molecular processes by which hormone-receptor complexes may modify membrane localized enzymes (for example, adenylate cyclase, guanylate cyclase, and phosphodiesterase), alter permeability barriers, or release yet unrecognized chemical mediators. We welcome comments and criticisms and we encourage Butcher *et al.* (*8*) as well as others to communicate directly with us concerning any aspect of our work which may need further clarification and which would mutually assist in our understanding of these scientific problems.

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Glaciers and Nutrients in Arctic Fiords

Apollonio (*1*), in his study of the effects of glaciers in Arctic fiords on the quantities of nutrients in the upper layers, has shown that the two fiords studied in Ellesmere Island, South Cape Fiord and Grise Fiord, differ in the quantity of nitrates in the upper layer. He ascribes this difference to the erosive effects of the glacier in South Cape Fiord in bringing nitrates to the fiord water. It seems to me, however, that Apollonio's evidence favors an upwelling effect of the glacier rather than an erosion effect. Both fiords contain similar concentrations of nitrates in their deeper waters (South Cape Fiord is considerably shallower than Grise Fiord). It is only in the upper layers, in the water with a density (σ_t) less than 26.8, that the difference in nitrate concentration is apparent. This suggests that the effect of the glacier is induction of upwelling of the deeper water to the surface rather than erosion of rocks on land. The agency of glaciers (also of icebergs) in inducing upwelling at their sea faces was suspected early in the study of ice in seawater (*2*, *3*). Hartley and Dunbar (*3*) demonstrated quite unequivocally the upwelling effect at the glacier face.

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