small increase in the optical density ratio at 280/260 was found—from 1.12 in the original actomyosin to 1.23 in the supernatant. However, when ATP was used to dissociate the actomyosin, strong absorption persisted at 260 nm even after dialysis, and the 280/260 ratio was 0.35 and 0.57 in two experiments. Calculations showed that the ATP concentration should have been too low for significant absorption. Residual binding of ATP or ADP by the proteins is a likely explanation for this. A value of 1.66 has been reported for the 280/260 ratio for *Physarum* myosin free of actin (*3*).

From all the results taken together, we conclude that ultracentrifugation in the presence of ATP or pyrophosphate sedimented much of the actin fraction (as well as some impurities visible in Fig. 1a) while both myosin and short F-actin fragments remained in the supernatant. Removal of the ATP by dialysis allowed the actomyosin complex to reform, now with a higher ratio of myosin to actin. Our results are in agreement with recent findings by Hatano and Ohnuma (*3*). They also found free myosin in the supernatant fraction after comparable treatments, and it is possible that this accounts for some residual binding of nucleotide in our experiments.

The demonstration that *Physarum* actomyosin enriched in myosin displays a polarity similar to that of muscle actomyosin confirms the previous report of polarity for *Physarum* filaments reacted with HMM S-1, the subunit from rabbit muscle myosin which retains both actin-binding ability and the enzymatic activity of myosin, from rabbit striated muscle (*2*). This similarity between the two actomyosins suggests that part of the process of cytoplasmic streaming depends on events fundamentally similar to those occurring in muscle contractions. But the myosin component from *Physarum* remains soluble at low ionic strength (*1*, *3*), forming only small aggregates. The present findings show that *Physarum* myosin can aggregate in a tail-to-tail fashion at least when attached to actin. However, the aggregations seen clearly so far seem to be in a parallel direction. It will be interesting to see if antiparallel tail-to-tail aggregations can occur with *Physarum* myosin in the absence of actin (*10*).

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11. We thank Dr. S. Matacić for natural actomyosin from rabbit striated muscle, Mrs. S. Shane for *Physarum* cultures, Miss B. Herzog for assistance with photography, and Drs. A. Loewy and D. Kessler for discussions. Supported by the Sloan Foundation and in part by NIH grants A108732 and HE04385.

23 June 1970

Prolactin: Evidence That It Is Separate from Growth Hormone in Human Blood

Abstract. *A highly sensitive bioassay has been developed for prolactin, which uses a mammalian end organ and which is capable of measuring the hormone in unextracted human plasma. High prolactin activity, largely neutralizable with antiserum to human growth hormone, is stimulated along with high immunoassayable growth hormone by insulin-induced hypoglycemia. High prolactin activity, not neutralizable with antiserum to growth hormone, exists with low concentrations of plasma growth hormone in postpartum patients and patients with galactorrhea.*

Since the initial demonstrations that purified growth hormone extracted from human pituitaries possessed lactogenic activity (*1*), efforts have been made to isolate a human prolactin separate from growth hormone. Although fractions that have variable ratios of prolactin to growth hormone have been prepared (*2*), the two biological activities have never been completely separated, and doubt has been expressed that human beings possess a separate prolactin comparable to that of lower animals (*3*). Nevertheless, several lines of evidence—including the absence of acromegalic changes in most patients with galactorrhea (*4*), the presence of postpartum lactation in patients with isolated growth hormone deficiency (*5*), histological studies of human pituitaries from postpartum subjects (*6*), and histological and bioassay studies of a pituitary tumor from a patient with galactorrhea, who showed low growth hormone in extracts of the tumor and high prolactin that was not neutralizable with antiserum to growth hormone (*7*)—suggest that there may be a distinct human prolactin. The inability of the pigeon crop-sac assay to measure the hormone in blood without elaborate extraction procedures has delayed resolution of this problem. We report here findings based on a more sensitive bioassay for prolactin in human blood which indicate (i) that prolactin exists in man and can circulate independently of growth hormone and (ii) that human growth hormone as it circulates in blood is intrinsically lactogenic.

The assay we have devised (*8*) depends on the ability of prolactin to cause differentiation and milk secretion of mouse breast tissue in organ culture (*9*). Fragments of tissue from Swiss albino mice, pregnant for 8 days and bred in our laboratory, are incubated 4 days in medium 199 containing insulin (10 μg/ml) and hydrocortisone (20 μg/ml) in an atmosphere of 95 percent O_2 and 5 percent CO_2. After incubation, tissues are fixed and stained with hematoxylin, phloxine, and saffron. Ovine prolactin (NIH-S8, 28 unit/mg) added to the medium at concentrations ranging from 1 to 50 ng/ml produces clearly visible and distinct secretory changes that are graded on a scale of 0 to 4+ (Fig. 1). When pooled plasma without detectable prolactin activity, from normal males, was added to the medium in 30 percent concentrations, it was found not to interfere with the response.

By partially or completely replacing this plasma with a sample to be tested, it has been possible to measure the prolactin concentration in circu-

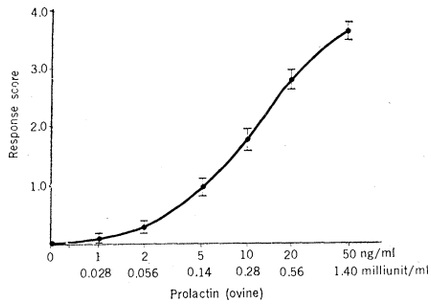

Fig. 1. Standard curve of combined experience with 44 assays in which ovine prolactin (NIH-S8, 28 unit/mg) was used as standard; 4.0 on the ordinate represents maximum secretory response. The vertical bars denote standard error of the mean.

lating plasma in a variety of conditions (*10*). The sensitivity of the system is 5 ng/ml (0.14 milliunit/ml), or somewhat better for ovine prolactin, and 15 ng/ml (0.42 milliunit/ml) for plasma samples at 30 percent concentration. The precision of the assay is inherently low but can be made satisfactory by multiple determinations at different dose levels. Specimens are routinely run in several assays; for most values reported here, which are not at the threshold of sensitivity of the assay, precision is approximately ± 25 percent. Specificity is high: no hormone tested in this system, including estradiol, progesterone, testosterone, adrenocorticotropic hormone, thyroid-stimulating hormone, human chorionic gonadotropin, vasopressin, and oxytocin, has given a positive response except human growth hormone and human placental lactogen. Highly purified (Wilhelmi HS1103C and HS1142), as well as clinical grade, preparations of human growth hormone, have been strongly lactogenic, with potencies ranging from 61 to 72 percent by weight of ovine prolactin. Very recently Loewenstein *et al.* (*11*) have reported an assay for prolactin, with similar sensitivity to this one, in which they used mouse breast tissue in organ culture and the production of *N*-acetyllactosamine synthetase, measured radiochemically, as the end point.

Prolactin activity has been undetectable (< 15 ng/ml) to date, under resting conditions, in the blood of normal men and in all except one of 20 normal women. This subject, whose growth hormone was 17 ng/ml, measured by radioimmunoassay (*12*), had prolactin activity that was just detectable at 15 ng/ml ovine equivalents (0.42 milliunit/ml). When tested after insulin-induced hypoglycemia, however, all of ten normal women and four normal men had elevated prolactin activity, ranging from 15 to 50 ng/ml ovine equivalents. Growth hormone concentrations as measured by radioimmunoassay, initially less than 15 ng/ml in all subjects except the one referred to above, rose after administration of insulin to peak concentrations ranging from 17 to 50 ng/ml (Fig. 2). In a group of 16 acromegalic patients, all of whom had elevated concentrations of plasma growth hormone ranging from 13 to 180 ng/ml, prolactin activity was detectable in all, with concentrations from 15 to 400 ng/ml ovine equivalents (Fig. 2). In smaller numbers of subjects whose plasma growth hormone had been elevated after exercise, administration of estrogen, and administration of arginine, we have also been able to detect prolactin activity. In every situation we have so far encountered in which immunoassayable growth hormone has been 15 ng/ml or greater, prolactin activity has also been detectable in plasma.

High concentrations of circulating prolactin, ranging from 15 to 130 ng/ml ovine equivalents, with normal or low concentrations of growth hormone (less than 0.3 to 5.0 ng/ml) were found by this method in ten lactating patients, 1 to 6 days postpartum; in seven women with galactorrhea of varying etiology; and in four endrocrinologically normal men and women receiving chlorpromazine or imipramine in high doses (*10*). A potent antiserum to human growth hormone (*13*), when preincubated at 1:10 dilution for 1 to 2 hours with patients' serum before assay, was found to be completely ineffective in neutralizing the plasma prolactin activity in any of the patients so far tested from these three categories. Under identical conditions of antiserum concentration and preincubation, the prolactin effect of human growth hormone preparations added to normal serum in high concentrations (500 ng/ml) is completely neutralized. We conclude that in these patients' plasma a prolactin molecule circulates which is different from human growth hormone.

In contrast to the above patients, the normal subjects (see Fig. 2), whose prolactin and growth hormone concentrations were simultaneously elevated after administration of insulin, had prolactin activity that could be largely neutralized by antiserum to human growth hormone. Plasmas from 8 of these 14 subjects, with prolactin activities ranging from 15 to 50 ng/ml ovine equivalents, were preincubated with antiserum to growth hormone at 1:10 dilution. Prolactin activity fell in all cases, becoming undetectable in two subjects and just detectable by unusually sensitive assays at approximately 6 ng/ml in three. In the remaining two subjects, who had the highest prolactin activities of 45 and 50 ng/ml, prolactin after neutralization was 20 and 17 ng/ml, respectively.

The reduction of prolactin activity after antibody treatment in these subjects with elevated growth hormones,

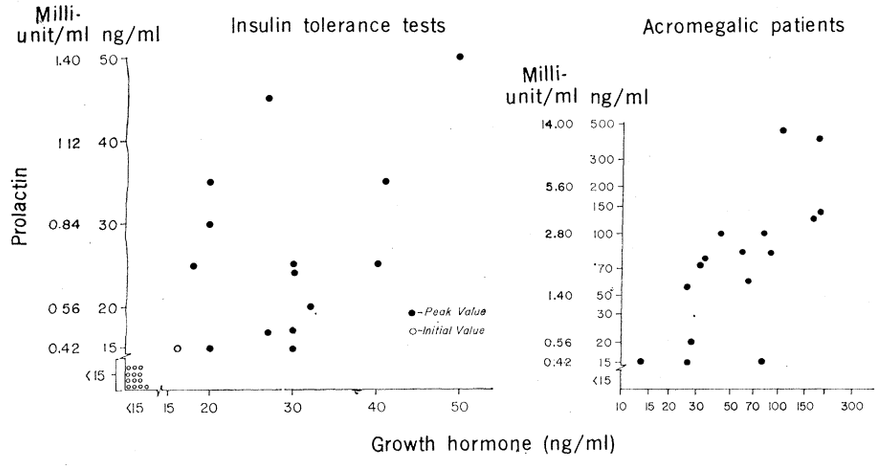

Fig. 2. (Left) Plasma prolactin activity plotted against growth hormone in same sample for initial (open circles) and peak values (closed circles) in 14 normal subjects during insulin tolerance tests. (Right) Plasma prolactin activity versus growth hormone in 16 acromegalic subjects. Note use of logarithmic scales on coordinates in this part of figure.

as contrasted with the absence of antibody effect in the postpartum patients and patients with galactorrhea whose growth hormone was not elevated, we interpret as being due to an intrinsic lactogenicity of the circulating growth hormone molecule. The persistence of some prolactin effect after exposure to antiserum, even though the latter was present in amounts sufficient to neutralize very large quantities of growth hormone, suggests that prolactin is released in some individuals concomitantly with growth hormone after induction of hypoglycemia by administration of insulin. This view is strengthened by the fact that the ratio of prolactin to growth hormone in the plasma of these subjects, as indicated in Fig. 2, although quite variable, is in many cases considerably above the highest ratio we have observed for any growth hormone preparation.

The high ratio of prolactin activity to growth hormone encountered in some patients with acromegaly (Fig. 2) similarly leads us to believe that prolactin as well as growth hormone may be secreted in this condition. In the one patient we have so far studied with antiserum, whose growth hormone was 150 ng/ml and whose prolactin activity was 400 ng/ml ovine equivalents, the prolactin activity after neutralization with antiserum to growth hormone was still markedly elevated at 161 ng/ml.

These studies provide evidence that a human prolactin molecule exists which circulates in human blood and is distinct from growth hormone. Ratios of the two hormones may differ widely in different conditions. These studies also indicate that growth hormone as it circulates in blood, like the material extracted from human pituitaries, possesses intrinsic lactogenic activity.

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Ribonuclease-Inhibitor System Abnormality in Dystrophic Mouse Skeletal Muscle

Abstract. *Skeletal muscle extracts from mice with muscular dystrophy contain severalfold higher than normal levels of free alkaline ribonuclease II activity and none of the free ribonuclease inhibitor normally present. This abnormal pattern is not seen in heart or liver extracts from dystrophic mice.*

The etiology of the disease or diseases of muscle called "progressive muscular dystrophy" is as much a mystery today as it was when Duchenne described "pseudohypotrophic muscular paralysis" in humans (*1*). A useful animal model for research into this genetic disease is the mouse with dystrophia muscularis, a condition first described in 1955 (*2*). There is considerable biochemical evidence suggesting altered rates of protein and ribonucleic acid (RNA) turnover in skeletal muscle of such mice (*3*).

In studies of the role of several neutral hydrolases in the degradation of protein and RNA in skeletal muscle (*4*), we have discovered large increases of alkaline ribonuclease II activity in dystrophic mice. Alkaline ribonuclease II (*5*) normally is present in mammalian tissue extracts almost completely inhibited by a low molecular weight protein inhibitor which occurs in consider-

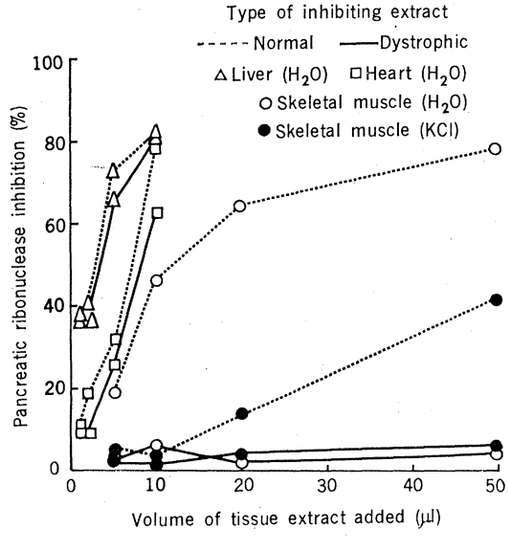

Fig. 1. Inhibition of pancreatic ribonuclease by extracts of normal and dystrophic mouse skeletal muscle, heart, and liver. Inhibitor assay reaction mixtures [modified from those of Shortman (*13*) and based on those for ribonuclease described in the legend to Table 1] consisted of 100 μl of assay buffer, enough water to give a final volume of 500 μl, 5 μl of pancreatic ribonuclease (1 μg/ml in 0.10 percent gelatin), the indicated amounts of tissue extracts, and after a 15-minute incubation at 0°C, 100 μl of RNA solution. Otherwise, assays and calculations were carried out exactly as described in the legend to Table 1. A small correction was made for the ribonuclease activity of added tissue extracts. The activity of pancreatic ribonuclease in an assay mixture containing no tissue extract was the control against which inhibition was measured. Inhibition by tissue extracts was expressed as the percentage of loss from this control value. The added pancreatic ribonuclease had an activity of 400 milliunits. The range of KCl concentrations present in the experiments did not measurably affect the inhibition. Data shown are for liver and skeletal muscle from the same individual animal. Because of their small size, three normal or dystrophic hearts were extracted together for this experiment.