geneic virus-free Friend virus-induced tumor.[1]

The nature of the antigen(s) involved has yet to be determined but these could include the soluble antigen reported by Stück *et al.* (6). For the present it does not seem unreasonable to suggest that malignant transformation of cells by Friend virus, a late phenomenon in Friend leukemia, is preceded by the formation of new cellular antigen(s). Extracts of infected spleens presumably would then contain this antigen(s), which, when administered with adjuvant, would invoke immunity against isogeneic tumor transplants.

Summary. Although only a small percentage of adult BDF_1 mice inoculated with Friend virus developed progressive Friend disease, a transplantable reticulum cell sarcoma was established from the one mouse with massive hepatosplenomegaly. Transplantation resistance to the tumor was induced by vaccination with either iodoacetate-treated tumor cells or Friend virus in adjuvant, but not with Friend virus alone.

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Early and Late Effects of Growth Hormone on the Rat Diaphragm* (32756)

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Investigations of the mode of action of growth hormone in the stimulation of protein synthesis have failed to completely elucidate this problem. In brief, studies utilizing the diaphragm of the hypophysectomized rat have shown that growth hormone stimulates the uptake of some but not all amino acids (1). When growth hormone is given *in vivo,* RNA polymerase is stimulated after 12 hours (2) and RNA synthesis is stimulated (3). However, no stimulation of RNA synthesis has been detected when the hormone is added *in vitro* (4,5), and inhibition of RNA synthesis with actinomycin does not prevent the *in vitro* stimulation of protein synthesis (4). It has therefore not been possible to identify the mechanisms involved in growth hormone stimulation of protein synthesis *in vitro.*

It is of significance that the reported studies have only assessed protein synthesis during incubations of two hours or less. Hjalmarson and Ahren (6) have shown that amino acid uptake is stimulated during the first two hours of exposure of the diaphragm to growth hormone, but after three hours, no stimulation is seen. Similar results have been independently noted in our laboratory (7), indicating that the short-term response of the diaphragm of the hypophysectomized rat differs significantly from the prolonged response. These findings, coupled with somewhat analogous findings by Goodman in studies on adipose tissue (8,9,10) prompted this investigation of the late effects of growth hormone on amino acid uptake and protein synthesis.

Materials and Methods. Fifty-gm rats obtained from the Charles River Breeding Laboratories and hypophysectomized at age 20 days, were killed by ether anesthesia and surgery, 2–3 weeks after operation. The intact hemidiaphragms were obtained with one of each pair serving as control, and incubation was carried out in the presence or absence of growth hormone (NIH-B7 and B12) in

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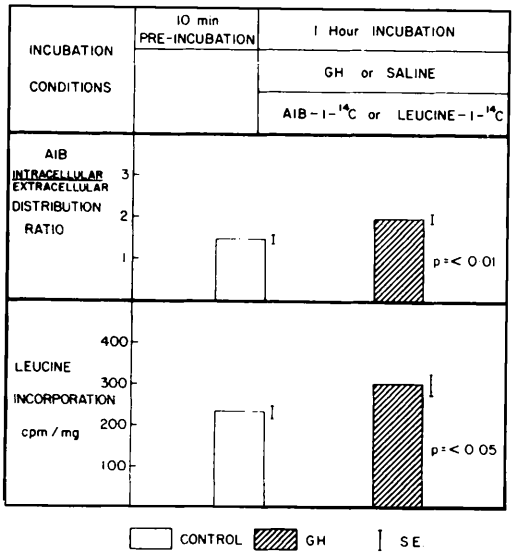

FIG. 1. Each hemidiaphragm was preincubated in 4 ml of KRB containing 2.5 mg.ml glucose for 10 min, then transferred to fresh medium in the presence or absence of BGH (25 μg/ml) and AIB-1-^{14}C (final conc. 0.05 mM, 0.04 μC/ml) or L-leucine-1-^{14}C (final conc. 0.1 mM, 0.1 μC/ml) for 1 hour.

Krebs–Ringer bicarbonate (KRB) buffer, pH 7.4, containing 2.5 mg/ml glucose. Alpha-amino-isobutyric acid (AIB-1-^{14}C, 0.05 mM, 0.04 μC/ml), or L-leucine-1-^{14}C (0.1 mM, 0.1 μC/ml) was added as indicated, the former being utilized in studies of amino acid accumulation and the latter in studies of amino acid incorporation into protein. In some studies, actinomycin D (kindly supplied by Merck, Sharp and Dohme, Montreal) was added at a concentration of 10 μg/ml medium.

Four incubation systems were utilized. Short-term (1 hour) incubations were carried out with and without growth hormone but without actinomycin D and similar incubations in the presence of this antibiotic. In addition, long-term (4 hour) incubations with and without growth hormone, and 4-hour incubations in the presence of actinomycin were performed. Incubation was carried out in a metabolic shaker at 37°C and all flasks were gassed with CO_2:O_2; 5%:95%, during the entire incubation.

After incubation, the tissues were rinsed, trimmed, weighed, and extracted as previously outlined (4), except that external standards were used during isotope counting in a Packard Tri-Carb Liquid Scintillation counter, and total protein in the counted aliquots was determined by weighing the planchets containing the extracted protein. Calculations of cellular space were also carried out as previously described.

Results. When the hemidiaphragm of the hypophysectomized rat was incubated in the presence of growth hormone for 1 hour, the accumulation of AIB (expressed as the intracellular–extracellular distribution ratio) was stimulated (Fig. 1). Similarly, protein synthesis as indicated by leucine incorporation was also stimulated (130%). In the presence of actinomycin at a concentration sufficient to produce a 98% inhibition of protein synthesis (4), growth hormone still stimulated both AIB accumulation and leucine incorporation (Fig. 2).

However, when intracellular amino acid accumulation and amino acid incorporation into protein were assessed after exposure of the tissue to growth hormone for 3 hours,

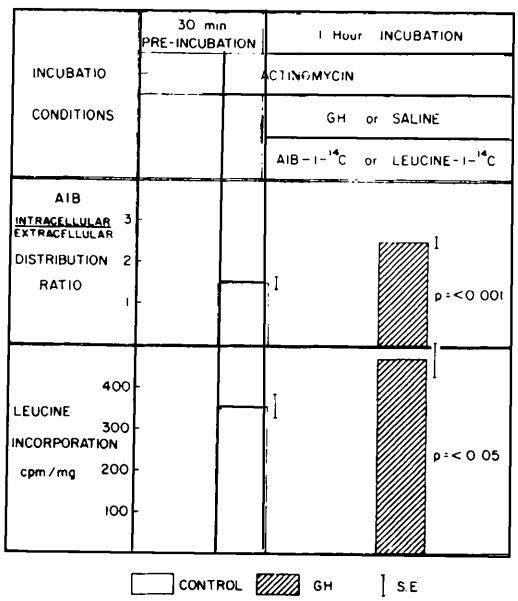

FIG. 2. Incubation conditions were as in Fig. 1, except that actinomycin was present in all flasks at a concentration of 10 μg/ml, and the preincubation time was 30 min.

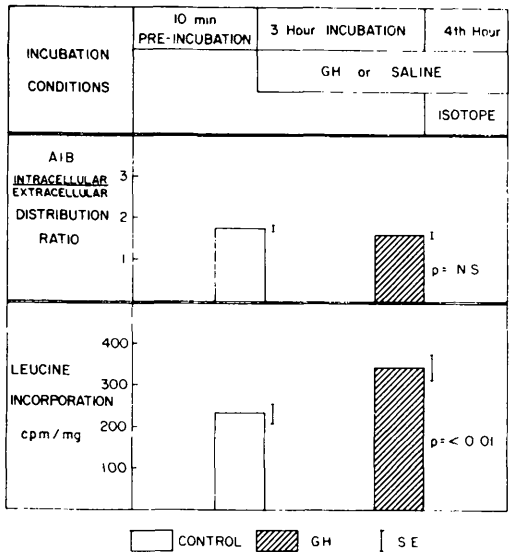

FIG. 3. Each hemidiaphragm was preincubated in KRB containing glucose, 2.5 mg/ml for 10 min, then transferred to fresh medium in the presence or absence of BGH (25 μg/ml) for 3 hours. For the fourth hour, each hemidiaphragm was transferred to fresh medium containing the same ingredients as previously present but now also containing AIB or L-leucine as in Fig. 1.

it can be seen that growth hormone did not stimulate amino acid accumulation while a stimulation of incorporation was still noted (Fig. 3).

Finally, when RNA synthesis was blocked by actinomycin in long-term incubation studies (Fig. 4), the stimulation of amino acid accumulation noted in the first hour was again seen. Protein synthesis, however, was not stimulated by growth hormone after prolonged inhibition of RNA synthesis.

Discussion. The results of these studies of growth hormone action in the first hour of *in vitro* incubation essentially serve to confirm findings already reported; namely, that growth hormone stimulates the intracellular accumulation of AIB; that protein synthesis is also stimulated, and that inhibition of RNA synthesis does not inhibit the growth hormone stimulation of protein synthesis.

In addition, it is also evident that inhibition of RNA synthesis has no effect on the short-term stimulation of amino acid uptake due to growth hormone. These data would lead to the conclusion that growth hormone acts principally at the level of the cell membrane in the stimulation of protein synthesis. Yet other evidence in the literature casts doubt on this conclusion, as it has been shown that active intracellular transport of amino acids is not required for growth hormone stimulation of protein synthesis (11), that stimulated protein synthesis occurs in the absence of any stimulation of amino acid uptake into the cell (4), and that stimulated protein synthesis may be dependent upon altered ribosomal function (3). Moreover, the findings reported here and also by Hjalmarson and Ahren (6) that enhanced amino acid uptake is only a transient finding also tends to indicate that enhanced amino acid uptake may not be the only significant physiological response to growth hormone. The long-term effects serve to verify this conclusion. It is possible to confirm the findings of Hjalmarson and Ahren (6) that no stimulation of amino acid uptake is seen after a 3-hour exposure to growth hormone. In addition, it is noted that at that time, in the absence of an increased amino acid transport, a hormonal stimulation of incorporation is seen.

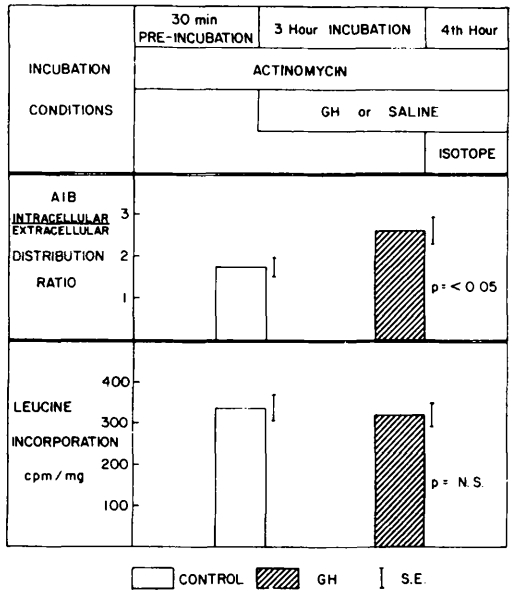

FIG. 4. Incubation conditions were as in Fig. 3, except that actinomycin was present in all flasks at a concentration of 10 μg/ml, and the preincubation period was 30 min.

The more noteworthy finding, however, is that inhibition of RNA synthesis abolishes any growth hormone stimulation of protein synthesis in the long-term incubation, a finding directly opposite to the observations in short-term incubations. This finding leads to the conclusion that prolonged growth hormone stimulation produces enhanced protein synthesis by a different mechanism than short-term stimulation, as the former is dependent on RNA synthesis. In addition, an intracellular regulatory mechanism may exist, mediated by RNA and protein synthesis, which controls amino acid uptake, for when actinomycin abolishes RNA synthesis, the enhanced uptake of amino acid noted in the short-term incubations continues for a prolonged period.

It must be pointed out that the metabolic activity of the diaphragm preparation appears unchanged after prolonged incubation, since the rate of amino acid incorporation remains the same, and comparison of CO_2 production in the first and fourth hours reveals no significant differences. In addition, the finding that CO_2 production is linear during prolonged (6 hour) diaphragm incubations (12) supports this observation.

It is unlikely that the prolonged exposure of the diaphragm to the toxic effects of actinomycin selectively impairs the ability of the diaphragm to respond to a stimulating hormone as one would also expect a fall in the control level of protein synthesis, a finding which was not observed. However, one must recognize that the antibiotic may produce other metabolic effects unrelated to protein synthesis which could affect these findings.

It thus appears that the long-term stimulation of protein synthesis by growth hormone is dependent upon RNA synthesis, and that RNA synthesis may also exert a regulatory effect on amino acid accumulation. However, the mechanism by which growth hormone stimulates protein synthesis in acute, short-term incubations remains obscure.

Summary. The mechanism of growth hormone stimulation of protein synthesis in short-term and long-term incubations differs. In studies utilizing the diaphragm of the hypophysectomized rat, it is seen that in short-term incubations hormonal stimulation of amino acid entry into the cell and incorporation into protein occurs, neither of which are affected by abolition of RNA synthesis (by actinomycin). In long-term (4 hour) incubations, no stimulation of amino acid entry is seen while protein synthesis is stimulated, and the latter stimulation *is* dependent upon RNA synthesis. In addition, abolition of RNA synthesis in long-term studies results in a stimulation of amino acid entry as was seen in short-term studies.

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