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Bacterial Synthesis of Substance Similar to Human Chorionic Gonadotrophin (39407)

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Recently, Livingston and co-workers (1) described the isolation of an organism from urine of cancer patients which, when cultured *in vitro*, produced a substance immunologically related to human chorionic gonadotropin (HCG). A sample of this material obtained from Dr. Livingston and examined immunologically for the presence of HCG using the hemagglutination inhibition technique (2) indeed confirmed the observations reported by them and persuaded us to proceed further.

We requested and received cultures of the organism from Dr. Livingston, and we ourselves were able to isolate an organism, which secreted HCG-like material, from the urine of a terminal colon carcinoma patient (A. K.).

The following report presents our data confirming the provocative findings of this group.

Material and methods. Culture of the organisms received from Dr. Livingston, as well as that isolated from the urine of A. K., a patient terminal with colon carcinoma, followed the procedure of Livingston *et al.* (1). Harvesting of the fermentation was also according to these workers. Although the organism has been named *Progenitor cryptocides* by the Livingston group no such classification is described in "Bergey's Manual." We have made no attempt to further classify this organism.[1]

Solids obtained by acetone precipitation of the contents of the fermentation flasks were extracted with 0.1 *M* EDTA (pH 6.8) and centrifuged. The extracts were subjected to immunological assay for HCG by the following procedures: hemagglutination inhibition (2); radioimmunoassay (RIA) as described by Saxena *et al.* (3) using purified HCG labeled with I^{125} and an antibody raised to HCG beta-subunit and absorbed with the alpha-subunit of HCG in order to increase specificity.

The extracts were also examined for the presence of biologically active HCG by radioreceptor assay (RRA) (4) using highly purified HCG labeled with I^{125} and bovine corpora lutea membranes isolated by the procedure described by Saxena and co-workers (4). The effect of the bacterial "HCG" on mouse Leydig cell preparations was established through the cooperation of Dr. K. Sundaram at Rockefeller University (5).

One bacterial extract was further purified by chromatography on Concavalin A-sepharose as described by Dufau *et al.* (6).

To compare the effect of proteolytic enzyme on bacterial "HCG" and the human hormone, samples of each were incubated with trypsin (20 μg trypsin per I.U. HCG activity) for 6 hr at 37°, pH 7.5 in 0.1 *M* phosphate buffer. Residual hormone activity was determined by RIA as described above.

Results and discussion. Figure 1 presents radioimmunoassay data comparing highly purified HCG (NIH reference HCG) with the bacterial "HCG-like substance" elaborated in trypticase soy broth by cultures of the microorganism furnished by Dr. Livingston. Parallelism between the dose response curves for this isolate and pure HCG indictates a strong immunological relationship between the mammalian hormone and the bacterial substance, particularly since the antibody used was raised by immunization with the beta-subunit of HCG. Extracts of trypticase soy broth cultures of *Escherichia coli* and *Staphylococcus aureus* did not contain a similar substance. Additional evidence for the similarity between HCG and

[1] Preliminary attempts at identification of the organisms secreting the HCG-like material indicated that the culture obtained from patient A. K. was a gram-negative rod. Confirmation by fermentation criteria indicated the organism was *Escherichia coli*. The two cultures obtained from Dr. Livingston were both gram-positive cocci.

the bacterial product is found in the parallelism of the slopes of the curves obtained in the radioreceptor assay for purified HCG and the bacterial substance isolated from cultures of the organism obtained from Dr. Livingston, as well as the organism isolated in this laboratory from the urine of a terminal carcinoma patient (Fig. 2). Incubation of isolated mouse Leydig cells with the bacterial extracts stimulated production of testosterone by these cell preparations offering

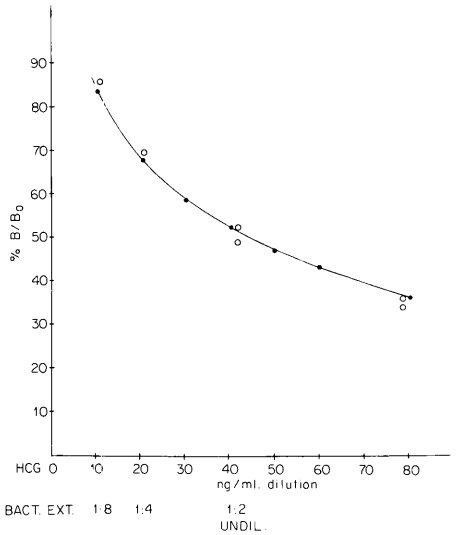

FIG. 1. Radioimmunoassay curves comparing the bacterial extract with human chorionic gonadotropin. ●, HCG; ○, bacterial extract.

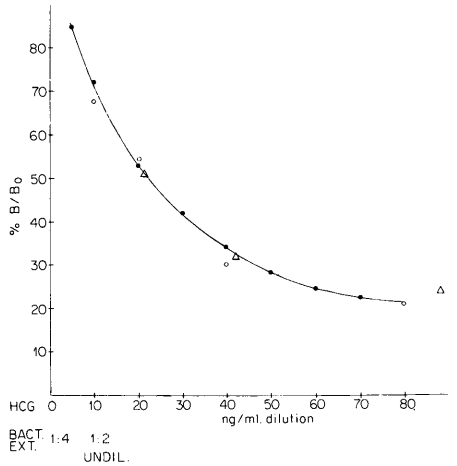

FIG. 2. Radioreceptor assay curves comparing purified HCG with bacterial extract. ●, HCG; ○, △, bacterial extracts.

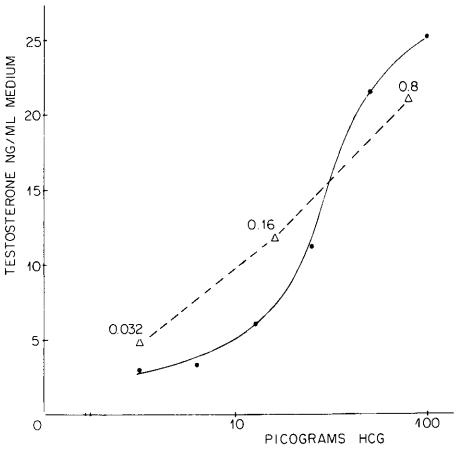

FIG. 3. Effect of purified HCG and bacterial extract on the production of testosterone by isogated mouse Leydig cell preparations. - - -△- - -△, Bacterial extracts (milliliter of extract); —●——●, purified HCG (picograms).

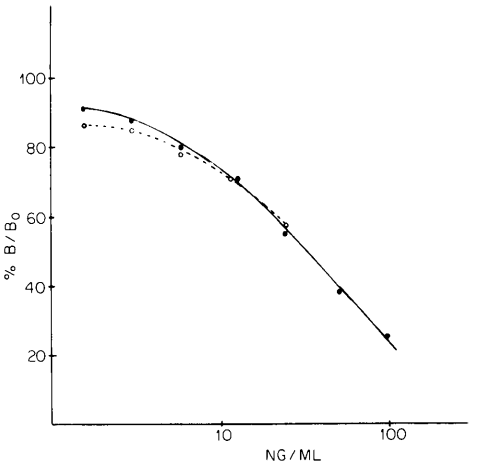

FIG. 4. Radioreceptor assay comparison of purified HCG with the 0.2 *M* methyl-alpha-D-mannopyranoside eluate, of the bacterial extract chromatographed on concanavalin A-Sepharose column. ——, HCG; -----, bacterial extract.

further indication of biological activity (Fig. 3).

These parameters (i.e., immunological and biological) characteristic of different conformational areas of the protein molecule present strongly convincing evidence for the similarity between the bacterial material and HCG.

Treatment of bacterial HCG with trypsin destroyed 38% of the activity as measured

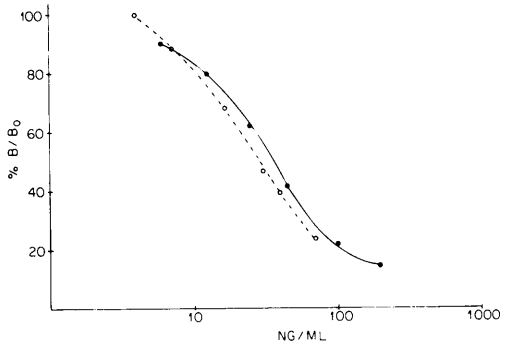

FIG. 5. Radioimmunoassay comparison of purified HCG with the 0.2 *M* methyl-alpha-D-mannopyranoside eluate, of the bacterial extract chromatographed on concanavalin A-Sephadex column. -----, bacterial extract; ——, HCG.

by RIA, a phenomenon also noted with purified HCG, and is indicative of the protein nature of the material. The probability that this substance is a glycoprotein is manifest in its adsorption onto Concanavalin A-Sepharose columns (Figs. 4, 5) from which 75% of the adsorbed bacterial extract could be eluted only with 0.2 *M* methyl alpha-D-mannopyranoside.

It should be pointed out that although the organism isolated from urine of patient A. K. elaborated HCG-like material when cultured, we were unable to demonstrate HCG in this urine by either RRA or RIA. It is also important to note that the amount of "HCG-like material" synthesized in culture is quite small and unpredictable, even when the same organism is employed. However, development of optimum culture conditions may remedy these difficulties and permit further work toward isolation, purification, and characterization of this substance.

We believe that the Livingston findings (1) are the first indication of the possible synthesis of a mammalian protein hormone by a microbial organism and imply either exchange of genetic material or the presence in microrganisms of genetic material capable of mediating such synthesis under particular conditions. However, until enough material is isolated and purified to enable work on structural and chemical properties, conclusions of strict identity should be withheld.

Summary. Our data tend to confirm the observations of Livingston *et al.* (1) that a microorganism(s) isolated from the urine of cancer patients is capable of producing a human chorionic gonadotrophinlike substance. Chromatographic properties as well as immunologic and receptor assay techniques offer strong evidence for this similarity.

We are indebted to Dr. K. Sundaram for his examination of the extracts for *in vitro* gonadotrophic activity, and to Drs. M. Goore and N. Starkovsky for their aid in performing the radioimmunoassay and radioreceptor assay. Also, to Dr. Livingston for supplying us with cultures of the organism as well as samples of her isolates.

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