Characterization and Properties of a New Placental Protein

PLACENTA is the source of several protein hormones described recently[1–4]. While working on a new method for making one of them, human placental lactogen, we found a new protein with hormonal properties, and we describe here its preparation and characterization.

A recently eliminated, membrane-free human placenta was homogenized in a Waring blender, freeze-dried, ground and extracted overnight with 450 ml. of 0·1 N acetic acid at 4° C. The supernatant was collected by centrifugation at 1,500 r.p.m. for 15 min and then freeze-dried. One hundred mg of it was then dissolved in 4 ml. of 0·1 N acetic acid and then chromatographed in a 'Sephadex *G*-75' column of 100 × 1 cm; 0·1 N acetic acid was used as eluent and 3 ml. fractions were collected in an automatic fraction collector.

Protein was assayed in each fraction by the method of Lowry[5]. Two principal peaks of protein were obtained, *A* and *B*, as shown in Fig. 1; the protein fractions thus obtained were tested against antiserum anti-HGH according to the immunodiffusion technique of Ouchterlony[6]; only the second peak showed a positive reaction of partial identity with the HGH prepared by the method of Raben[7]. This second peak could possibly correspond to the human placental lactogen described by Josimovich and MacLaren[3] and Kaplan and Grumbeck[4].

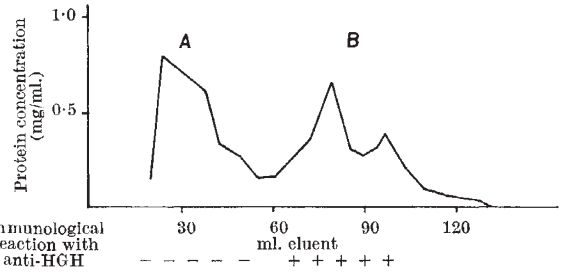

Fig. 1. Gel filtration on 'Sephadex *G*-75' of a placental protein extract.

The protein fraction contained in peak *A* (Fig. 1) was concentrated by freeze-drying and redissolved in the appropriate solvent for each of the following experiments.

The purity of this protein fraction was established by electrophoresis on cellulose acetate strips. The lyophilized protein was dissolved in 0·33 M boric acid–0·1 M LiOH buffer *p*H 8 and submitted to electrophoresis in the same buffer for 90 min at 25 V/cm; protein was then stained on a 0·5 per cent solution of water-soluble nygrosin in methanol–acetic acid–water 50 : 10 : 40 (ref. 8). Only one band of protein was obtained with a mobility of +3 cm. Analytical chromatography in 'Sephadex *G*-100' using 0·05 M *tris* buffer *p*H 8 as solvent and eluent also revealed only one symmetrical peak of protein with a molecular weight of approximately 70,000 according to the method of Andrews[9].

Comparative studies with HGH, ACTH, HPL, CGT and TSH and albumin were made in order to establish the distinct identity of peak *A*. Zone electrophoresis, immunodiffusion, immunoelectrophoresis as well as haemagglutination showed non-identity with all these protein hormones; we were able to show that cross-contamination was less than 1 per cent.

The injection of peak *A* dissolved in saline, in doses of 30 μg/day to young female rats, produced a significant increase in uterus weight ($P = 0{\cdot}001$). This effect was completely inhibited by the simultaneous injection of antiserum anti-peak *A* prepared in rabbits, as shown in Fig. 2.

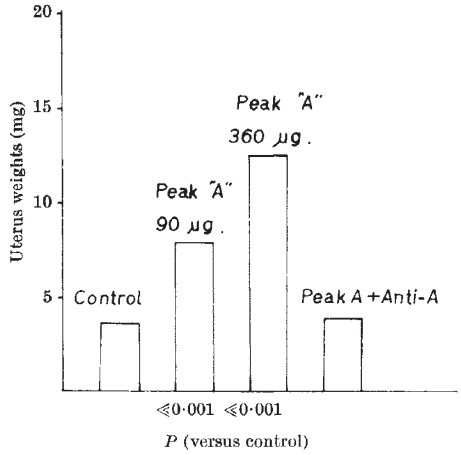

Fig. 2. Action of the placental protein and its antiserum on the weights of the uterus of rats.

The results of these preliminary experiments towards the hormonal activity of our protein fraction lead us to suggest the provisional name of "placental uterotrophic hormone" for this new placental protein. The physiological role of the protein is being investigated in our laboratory.

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[1] Beas, F., and Flores, H., *Third Intern. Cong. Endocrinol.*, Mexico, No. 157, 200 (1968).
[2] Beas, F., Salinas, A., and Pak, N. (in the press).
[3] Josimovich, I. B., and MacLaren, J. A., *Endocrinology*, **71**, 209 (1962).
[4] Kaplan, S. L., and Grumbeck, M. M., *J. Clin. Endocrinol. Metab.*, **24**, 80 (1964).
[5] Lowry, O. H., Rosebrough, N. I., Farr, A. C., and Randall, R. I., *J. Biol. Chem.*, **193**, 265 (1951).
[6] Ouchterlony, O., *Acta Path. Microbiol. Scand.*, **32**, 231 (1953).
[7] Raben, M. S., *Science*, **125**, 883 (1957).
[8] Barrett, R. I., Friesen, H., and Astwood, E. B., *J. Biol. Chem.*, **237**, 432 (1962).
[9] Andrews, P., *Biochem. J.*, **91** (2), 222 (1964).

Control of Cholesterol Biosynthesis by a Plasma Apo-lipoprotein

MEVALONIC acid (MVA) is well known as a precursor of cholesterol[1], and it is also established that squalene is an intermediate in the biosynthesis of cholesterol[2,3]. When liver microsomes plus the cell sap are incubated with 2-^{14}C mevalonic acid in air, most of the radioactivity is incorporated into cholesterol and very little radioactivity is associated with squalene. Conversely, in anaerobic conditions squalene accumulates[4,5]. We have encountered an interesting situation in which squalene accumulates during cholesterologenesis in aerobic conditions.

A rat liver homogenate was prepared in Bucher's medium[6], and centrifuged at 18,000*g* for 20 min. The 18,000*g* supernatant containing microsomes plus the cell sap was incubated with 2-^{14}C DL-MVA (Radiochemical Centre, Amersham), NADPH generating system, NADH, ATP generating system and reduced glutathione at 37° C in air for 1–1·5 h. In these incubations about 30–40 per cent of the 2-^{14}C DL-MVA was converted to sterols—the L-isomer of mevalonic acid is not incor-