Effect of the Vehicle in the Bioassay of Human Chorionic Gonadotrophin

Many authors[1-5] have reported that the biological procedures for the estimation of human chorionic gonadotrophin (HCG) in serum yield unreliable data owing to the interference of some constituents of serum. However, DICZFALUSY and LORAINE[6] claim that the activity of HCG is not significantly increased by plasma if the bioassay used is based on the prostatic weight and seminal-vesicle weight of intact immature rat[7,8]. On the contrary, we have shown in previous papers[9,10] that, using this method, an enhancement of the hormonal activity occurs, which is presumably due to the plasma proteins. We have observed this effect even under conditions of extreme dilution of plasma (0.0004 ml plasma/100 ml saline) where a 1:1 ratio between plasma molecules and hormonal molecules is approximately attained.

According to the most widely accepted hypothesis, the effect of plasma proteins is a protection of hormone against its destruction in the body of the animals after injection. In the present work, we have tested the effects of various single plasma proteins and of other high molecular weight substances of both protein and non-protein nature.

For the bioassays, HCG (3,560 IU/mg, Ormonoterapia Richter, Milano) was dissolved (2 IU and 4 IU in 3 ml) in the following diluents: a) saline solution; b) 1% bovine serum albumin (Behringwerke) in saline; c) 1% bovine serum α-globulin (Fluka)[9] in saline; d) 1% bovine serum β-globulin (Fluka) in saline; e) 0.8% bovine serum γ-globulin (Behringwerke) in saline; f) 0.7% bovine fibrinogen (Behringwerke) in saline; g) 1% ovalbumin in saline; h) 1% casein in saline; l) 3.5% polyvinylpyrrolidone (PVP) in isotonic solution (Subtosan Farmitalia); m) 6% depolymerized dextran (M.W. $\simeq$ 80,000) in saline (Macrodex Baxter).

The bioassay (based on the weight of the ventral prostate and seminal vesicles in immature male rat) and the statistical analysis were carried out according to DICZFALUSY[7,8]. The results are shown (Table) as relative potencies in terms of saline solution of hormone. It should be noted that: 1. The enhancing effect of albumin and β-globulin is comparable with that produced by the whole plasma (about 2.5 times[11]). A potentiating effect on the activity of the HCG by serum albumin in the ovarian ascorbic acid depletion test has been reported recently[12]. 2. The interpretation of the effect of α-globulin is ambiguous because of its contamination by β-globulin. 3. Ovalbumin shows an effect similar to that of serum albumin. 4. Casein is less effective, whilst PVP and dextran have little or no effect.

Relative potencies of HCG solutions in different diluents

Diluent	Relative potency
1% bovine serum albumin	2.63
1% bovine serum α-globulin	2.15
1% bovine serum β-globulin	2.67
0.8% bovine serum γ-globulin	1.37
0.7% bovine fibrinogen	1.42
1% ovalbumin	2.33
1% casein	1.52
3.5% PVP (Subtosan)	1.29
6% depolymerized dextran (Macrodex)	0.86

Assuming that the enhancing effect of the plasma proteins is due to binding of a HCG molecule by a protein molecule, albumin, β-globulin and perhaps α-globulin should be responsible for this binding. Our assumption is supported by the report by BOURRILLON[13] that the pregnant mare's serum gonadotrophin (PMSG) also forms a stable 1:1 complex with a plasmatic acid α_1-glycoprotein.

Among other tested non-plasmatic macromolecules, only ovalbumin, in some respects similar to serum albumin, seems capable of such binding.

Riassunto. L'albumina serica, le β-globuline e, forse, le α-globuline presenti nel mezzo di iniezione, incrementano notevolmente l'attività della gonadotropina corionica umana sugli organi sessuali accessori del ratto maschio impubere. Anche l'ovalbumina esercita lo stesso effetto.

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[1] W. O. MADDOCK and R. B. LEACH, J. Lab. clin. Med. *40*, 923 (1952).
[2] W. O. MADDOCK, I. TOKUYAMA and R. B. LEACH, Proc. Soc. exp. Biol. Med. *84*, 352 (1953).
[3] B. LUNENFELD, E. RABAU, A. HARELL-STEINBERG and A. SZEJNBERG, Acta endocr. *24*, 113 (1957).
[4] R. BORTH, B. LUNENFELD and H. DE WATTEVILLE, Acta endocr. *24*, 119 (1957).
[5] V. INSLER and B. LUNENFELD, J. Endocr. *31*, 151 (1965).
[6] E. DICZFALUSY and J. A. LORAINE, J. clin. Endocr. Metab. *15*, 424 (1955).
[7] E. DICZFALUSY, Acta endocr. *17*, 58 (1954).
[8] E. DICZFALUSY, L. NILSSON and A. WESTMAN, Acta endocr. *28*, 137 (1958).
[9] Cellogel electrophoresis of the commercial product revealed a small contamination by β-globulin.
[10] G. LUGARO, G. GIANNATTASIO and M. M. CASELLATO, Folia endocr *21*, 243 (1968).
[11] G. GIANNATTASIO, G. LUGARO and E. MANERA, Folia endocr. *21*, 771 (1968).
[12] R. BORTH and A. MENZI, Acta endocr. *61*, 89 (1969).
[13] R. BOURRILLON, R. GOT, J. BOUGUEREAU and R. MARCY, Bull. Soc. Chim. biol. *39*, 1119 (1957).

The Effects of Seed Extracts on Local Lesion Formation by *Tobacco necrosis* Virus

Although seed transmission of viruses is a comparatively rare event, it is nevertheless, of enormous economic importance particularly as widespread distribution of plant diseases may occur by this method. The question as to why highly infectious viruses are not transmitted more readily via seeds remains unanswered. It has been suggested that viruses may be excluded from seed tissues[1,2], or that viruses may be unable to maintain themselves in gametophytic tissue[3]. Inactivation of virus by compounds within seed tissues has also been considered as a possible means of virus elimination from seeds[4-7]. Little information on the inhibition of viruses by seed extracts is available however. In order to study the possible inhibition of virus multiplication by seed extracts