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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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

a variety of seeds were ground in distilled water and the resulting slurries centrifuged at 3000 *g* for 15 min to remove cell debris. Such extracts were mixed with equal volumes of *Tobacco necrosis* virus (TNV) suspensions prepared in 0.06 *M* phosphate buffer pH 7.0. Inoculations were made immediately onto French bean leaves (*Phaseolus vulgaris* var The Prince) using carborundum as an abrasive. Control plants were inoculated with similar samples of buffered TNV diluted with water.

Activity Quotients were clcaulated for each treatment using the method of BENDA[8].

$$\text{Activity quotient} = \frac{\text{Number lesions on treated leaves}}{\text{Number lesions on control leaves}}$$

Each experiment was replicated on at least 2 occasions. Control plants showed between 30 and 40 local lesions per leaf. The Table shows that of the 14 seeds examined 10 reduced the number of local lesions formed on test plants resulting in activity quotients less than unity. Inhibition of lesion number was most marked in the case of *Brassica napus* (Rape.), *Beta vulgaris* (Beet) and *Beta vulgaris* var. *Rapa* (Sugar Beet). Partial reversal of inhibition was brought about by heating the seed extracts to 100 °C for 10 min. No changes in size or rate of lesion development could be detected.

Effects of seed extracts on the local lesion production by *Tobacco necrosis* virus

Seed extract	Activity quotient Unheated extract	Heated extract[a]
Lactuca sativa, L.	2.40	1.10
Lycopersicum esculentum, Mill.	0.33	0.87
Brassica napus, L.	(0)[b]	0.18
Brassica alba, Rabenh.	0.26	0.56
Raphanus sativus, L.	0.80	1.07
Cheiranthus cheiri, L.	0.26	0.40
Papaver orientale, L.	0.60	0.40
Chenopodium amaranticolor, Coste and Reyn.	0.33	0.66
Beta vulgaris, L. (Beet)	0.06	0.41
Beta vulgaris var. *Rapa*, Dumort (Sugar-Beet)	0.11	0.46
Vicia faba, L.	4.00	[c]
Phaseolus aureus, Roxb.	2.00	[c]
Phaseolus vulgaris L. var. 'The Prince'	2.76	2.04
Nicotiana glutinosa L.	0.9	1.0

[a] Such extracts were heated to 100 °C for 10 min. [b] Complete inhibition of lesion formation. [c] Not tested.

Particularly interesting are the results obtained using extracts prepared from *Lactuca sativa* (Lettuce), *Vicia faba* (Broad bean), *Phaseolus vulgaris* (French bean) and from *Phaseolus aureus* (Mung bean). These extracts consistently yielded activity quotients greater than one, suggesting enhancement of virus activity. This enhancement was reduced but not completely eliminated by heating extracts to 100 °C for 10 min. Enhancement or augmentation of virus activity has not previously been described for seed extracts although such a phenomenon has been observed when plant saps were mixed with virus suspensions[8,9].

These preliminary results suggest that some seed extracts contain compounds favourable to virus multiplication – augmenters – as well as inhibitory compounds. It is interesting to note that the seeds of *Lactuca*, *Phaseolus* and *Vicia* known to be frequently involved in virus transmission contain augmenters. The ability of seeds to act as carriers of plant viruses may reflect differences in the concentration or activity of virus augmenters and inhibitors present in seed tissue. Thus virus entering seeds with predominantly augmentative properties would be seed transmitted whereas virus entering seeds with largely inhibitory properties would fail to be transmitted by seed. Further experiments to test this hypothesis and to identify the compounds involved are being undertaken.

Zusammenfassung. An Samen von *Lactuca sativa*, *Vicia faba*, *Phaseolus vulgaris* und *P. aureus* steigerte das Tabaknekrose-Virus Läsionen, hervorgerufen auf den Blättern der *P. vulgaris*. Zehn andere Samenproben widerstanden hingegen der Läsionsbildung.

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The Innervation of the Prothoracic Glands of *Cerura vinula* L. (Lepidoptera)[1]

Preliminary observations have so far not yielded much information on the innervation of the prothoracic glands, because the investigators contradicted one another (ARVY and GABE[2], SRIVASTAVA[3]). WILLIAMS[4] and HERMAN and GILBERT[5] reported innervation of the prothoracic glands on the lepidopterous silkmoth *Hyalophora cecropia* by classical cytological techniques, as did also SCHARRER[6] on the cockroach *Leucophaea maderae* in more detailed studies using the electron microscope. SRIVASTAVA and SINGH[7] found that the prothoracic glands of *Papilio demoleus* are innervated by nerves which form a network of nerve fibres closely surrounding each gland cell.

The prothoracic glands of *Cerura vinula* L. are band-like structures that lie on the large ventral trachea on either side of the oesophagus. By staining the nerves in situ, using the leucomethylene blue nerve staining technique of ZACHARUK (cit. STAY and GELPERIN[8]), I have found that the glands are not only linked with the prothoracic ganglia and the interganglionic connectives between the prothoracic and the metathoracic ganglia through nerves, but also with the suboesophageal ganglion. The nervous connections with this ganglion are very small and have not been observed to penetrate the gland in methylene-blue preparations (BÜCKMANN[9]).