Table 1. *Effect of foetal calf serum and cortisol on the synthesis of collagen and hyaluronic acid in human skin fibroblasts.*

Serum (%)	Collagen (d min^{-1} × 10^{-5})* Cortisol (M) 0	Collagen Cortisol (M) 10^{-5}	Hyaluronic acid (d min^{-1} × 10^{-5}) Cortisol (M) 0	Hyaluronic acid Cortisol (M) 10^{-5}
0	79·0 (74·2–82·1)**	80·3 (68·1–108·2)	12·3 (15·2–10·3)	4·8 (4·7–5·2)
10	132·3 (127·4–142·8)	148·5 (139·2–157·4)	124·4 (113·2–132·0)	57·7 (51·2–63·4)

* Measured as [^{3}H]hydroxyproline.
** Mean and range; n = 5–7 in collagen, n = 3 in hyaluronic acid.

was about ten times higher in the presence of 10% foetal calf serum.

Effects of cortisol on the cell layer hyaluronic acid, sulphated GAGs and collagen were not studied, because earlier experiments (Doherty & Saarni, 1976; Saarni & Hopsu-Havu, 1977) had shown that they are parallel to the effects of cortisol on the synthesis of these compounds in the medium. It thus appears that in short term incubations 1×10^{-6} M cortisol significantly inhibits the synthesis of hyaluronate, but does not change the synthesis of DNA, sulphated GAGs or collagen. This suggests that reduced hyaluronate synthesis is a sensitive indicator of glucocorticoid action on normal human fibroblasts.

September 22, 1977

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A potential interaction between gentamicin and cephalexin

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Gentamicin is often administered with a cephalosporin, or a penicillin, as these combinations are considered to be synergistic (Watanakunakorn & Glotzebecker, 1974). But there is a possibility of an interaction, for example, visible precipitation in intravenous mixtures of gentamicin with cephalothin (Noone & Pattison, 1971) or with cloxacillin (Noone & Pattison, 1971) or with cephapirin (Prasad, Granatek & Mihotic, 1974) have occurred. Also, the antibacterial activity of gentamicin can be reduced by the presence of carbenicillin (Noone & Pattison, 1971; Riff & Jackson, 1972) and ticarcillin (Davies, Morgan & Anand, 1975; Ervin, Bullock & Nuttall, 1976) at high ratios (20:1 to 30:1) of β-lactam antibiotic to gentamicin. It is therefore necessary to ascertain if such an interaction is likely *in vivo*. The potential interaction between cephalexin and gentamicin has therefore been examined.

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Vapour pressure osmometry. Since the vapour pressure of a solution is dependent on the total number of molecules and ions of solute present, the technique provides a useful tool for the determination of the molecular weight of a solute in terms of all the species present (this is the number average molecular weight, $\overline{Mn}$). Thus the molecular weight of an ionized solute will be lower when determined by this technique than when

calculated from its chemical formula. The technique may therefore be used to determine the dissociation or association, in solution, of a compound of known molecular weight. Alternatively it may be used to determine the degree of binding between two compounds of known molecular weight. A Hewlett Packard Model 302B instrument was used to investigate the degree of complexation between cephalexin and gentamicin sulphate in aqueous solution. It consists of two thermistor beads connected to a Wheatstone bridge and suspended in a thermostatted chamber saturated with solvent vapour. After zeroing the null meter on the bridge with one drop of solvent on each bead, the solvent on the reference bead is replaced with one drop of the solution under investigation. The condensation of solvent vapour on the solution causes the temperature of the solution drop to rise. This is indicated by a deflection in the null meter which is corrected by adjusting the voltage. The system is allowed to equilibrate, and the final voltage V is recorded. A plot of V *vs* concentration of solute (C) gives a straight line passing through the origin and the slope (V/C) = K/$\overline{M}$n. The value of K is an instrument constant which can be calculated independently using mannitol, a non-interacting solute of known molecular weight. By this method $\overline{M}$n for cephalexin in water was found to be 353, which agrees within experimental limits with the theoretical molecular weight (347) and shows that cephalexin exists as a single species in water. Similarly the value of $\overline{M}$n for gentamicin sulphate in water was found to be 347. Only an approximation of the degree of ionization of gentamicin sulphate could be made as this drug is a mixture of antibiotics (mainly gentamicins C_1, C_2 and C_{1a}) present as their sulphates (sulphate ion constitutes 31–34% of gentamicin sulphate). From the molecular weight and relative proportions of the three major constituents (Cooper, Yudis & others, 1971), and the content of sulphate, the degree of ionization of gentamicin sulphate in water was calculated to be 77%.

Solutions containing known concentrations of cephalexin and gentamicin in an approximately 1:1 ratio were next prepared, and values of V obtained (Table 1). Linear regression of the plot of V *vs* C_T (weighting the origin by a factor of 10) gave a value of 406 for $\overline{M}$n. Even though the exact nature of gentamicin sulphate in solution is not known, it is possible, knowing the values of $\overline{M}$n for cephalexin and gentamicin determined independently, to predict a theoretical additive value of V (V_{TL}) for each mixture of the two drugs (Table 1). A plot of V_{TL} *vs* C_T yields a value of $\overline{M}$n = 349 and this assumes that no interaction occurs between the two drugs. The fact that the observed $\overline{M}$n is higher than the theoretical value shows that there are fewer independent molecules in solution and so the two drugs must interact to some degree. The system is complex, but the simplest case which complies with the experimental data is where one molecule of gentamicin base binds to two molecules of cephalexin, with 90% of the gentamicin not in a complex with cephalexin.

Table 1. *Concentrations (g kg^{-1}) of gentamicin sulphate, C_G, cephalexin, C_C, with theoretical and observed values of V (V_{TL} and V_{OBS}) for the additive concentration C_T.*

C_C	C_G	C_T	V_{TL}	V_{OBS}
4·487	5·305	9·792	68·06	55·53
4·256	4·147	8·403	58·35	49·85
3·063	3·235	6·298	43·75	42·05
1·965	2·501	4·466	31·05	30·03
1·169	1·062	2·231	15·48	14·75

Electrophoresis. Electrophoresis of gentamicin sulphate, cephalexin and a 1:1 mixture of the two drugs was used to confirm that a complex is formed between these two drugs. The support medium was Whatman No. 1 filter paper and the buffer was 0·05 M ammonium acetate –0·05 M ammonia pH 9·4. After 2 h at 250 V, cephalexin was visualized under light at 350 nm. Gentamicin and cephalexin were then visualized by spraying with ninhydrin solution. All migration distances were calculated with respect to cephalexin. Cephalexin migrated 1 unit towards the anode and gentamicin migrated 1·46 units towards the cathode. The mixture gave two fluorescent spots, one corresponding to cephalexin and the second at 0·51 units towards the cathode. The latter spot is therefore positively charged, possesses a chromophore and has a lower charge density than gentamicin and so can be assumed to be a complex of the two drugs. The intensity of fluorescence of this spot was much less than that of the spot corresponding to cephalexin, confirming that only a small fraction of the cephalexin is complexed with gentamicin.

Effect of the interaction on the antibacterial activity of gentamicin. Gentamicin has a low therapeutic index. The serum concentration should be maintained between 4 and 10 μg ml^{-1} (Reeves, 1974) yet ototoxic and nephrotoxic effects may be seen at peak serum concentrations of 12 μg ml^{-1} or minima (before next dose) of 2 μg ml^{-1} (Hewitt, 1974; Dahlgren, Anderson & Hewitt, 1975). The presence of an interacting drug might therefore reduce the efficacy or increase the toxicity of gentamicin. The effect of cephalexin on the antibacterial activity of gentamicin was therefore assessed at concentrations achieved in serum during therapy. An agar diffusion assay was used with *Pseudomonas aeruginosa* strain 10662 (NCTC) as the test organism. This organism is especially sensitive to gentamicin but is not inhibited by cephalexin. Since the presence of Ca^{2+} and Mg^{2+} decreases the sensitivity of antimicrobial assays of gentamicin (Gilbert, Kutscher & others, 1971) Mueller-Hinton broth was solidified with agar (1·5%) which had been dialysed ×3 against 1% EDTA and ×4 against distilled water. The resulting Mueller-Hinton agar was

found to contain only 0·05 μg ml^{-1} Ca^{2+} and no detectable Mg^{2+} (atomic absorption spectroscopy) and there was a sixfold increase in sensitivity of the assay for gentamicin using this agar. Agar seeded with organism was poured to give 2 mm plates. Four wells (11 mm diameter) were bored in each plate and filled with solution to be assayed. The plates were stood for 30–60 min to allow diffusion into the agar then incubated at 37° for 18 h and the zones of inhibition measured. Three ratios of cephalexin to gentamicin were used, 1:1 (the ratio used in the vapour pressure osmometry and electrophoresis), 5:1 (the ratio in serum during therapy) and 20:1 (since the degree of inactivation by carbenicillin increases as the ratio of β-lactam antibiotic to gentamicin increases, Riff & Jackson, 1972). All solutions were stored at 37° for 3–5 days before assay. The results ae summarized in Table 2.

Table 2. *Effect of cephalexin on the antibacterial activity of gentamicin.*

Gentamicin (base) μg ml^{-1}	Zones of inhibition[1] (mm) Gt[2]	G[2]	C:G (1:1)	C:G (5:1)	C:G (20:1)
Distilled water					
2·5	13·5	12·5	13	13·5	12·5
5·0	17	15·5	16	16·5	16·5
10·0	20	19·5	18	19·5	19·5
20·0	23	22·5	21·5	22·5	22
Sorensen's phosphate buffer (pH 7·8)					
2·5	14·5	15	14·5	14·5	14
5·0	17	17·5	16	16	16·5
10·0	18	18	18	17·5	17·5
20·0	20	20	19·5	19	19
Albumin (4% w/v)					
2·5		13·5	13·5	14·5	13·5
5·0		15·5	16	18	16
10·0		17·5	18·5	18	19
20·0		22	21·5	22	22
Citrated plasma					
2·5		16		16	
5·0		18·5		19	
10·0		19·5		20·5	
20·0		22·5		22·5	

[1] *vs Pseudomonas aeruginosa* strain 10662, on Mueller-Hinton Agar prepared with dialysed agar; each value is a mean of 2 readings.
[2] Gt denotes freshly prepared gentamicin solution, G and C denote gentamicin and cephalexin the solutions having been stored at 37° for between 3 and 5 days.

On storage of gentamicin and on mixing with cephalexin there is an overall slight decrease in the zone of inhibition, but in none of the media was the observed decrease greater than 10%, whereas the limits of the microbiological assay are, at best, ±15% (Reeves, 1974). The presence of cephalexin therefore has no measurable effect on the antibacterial activity of gentamicin when assayed by the agar diffusion method. In practice other organisms such as *Staphylococcus aureus* are often used as test organism with a β-lactamase added to inactivate the β-lactam antibiotic. However, no effect on the assay of gentamicin was seen on adding β-lactamase, or with cephalexin and β-lactamase when *S. aureus* was used as test organism (nutrient agar, 6 mm wells).

In conclusion, although there is formation of a complex between cephalexin and gentamicin, this has no measurable effect on the antibacterial activity of gentamicin. The microbiological assay will therefore give a true reflection of the plasma concentration of gentamicin when this drug is given in combination with cephalexin.

July 29, 1977

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