Immediately upon receipt of ICR mice (Mogul Corp.), they were infected with 10 *H. nana* cysticercoids and drug therapy was inaugurated after 14 days with the first appearance of eggs in the feces. The high recovery of worms in the control and 50 mg/kg groups at autopsy is indicative of animal room reinfections, particularly since most of the parasites recovered were immature. At 100 mg/kg, worms were recovered from only 1 animal.

CGP 4540 had no demonstrable effect upon either *S. mansonoides* egg counts or worm recovery when CGP 4540 was administered to the host cats at dose levels up to 150 mg/kg.

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## Effect of aspirin and vitamins C and E on synovial rheumatoid arthritic and other cells[1]

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*Summary.* Normal and rheumatoid arthritic human synovial cells, normal rat muscle and bone cells, were cultured with combinations of aspirin (acetylsalicytic acid), vitamins C and E. Aspirin reduced percent growth of all cells by about 1/5 relative to controls. High vitamin C eradicated arthritic cells. In combinations, vitamin C was most important in eradicating arthritic cells. A low-aspirin, low-vitamin C combination was most effective in reducing arthritic cell populations, while having little effect on normal cells. Vitamin E retarded but did not prevent the action of vitamin C.

Amongst many effects aspirin (acetyl-salicytic acid) is believed to inhibit prostaglandin synthesis[2,3]. Prostaglandins are believed to be modulators of the activity of the body[4], perhaps mainly involved in local communication between cells, especially in defensive reactions induced by damage or stress. Aspirin is an anti-defensive drug inhibiting many of the reactions of the body to noxious agents. The potency of aspirin relative to sodium salicylate differs in different sites and for other reasons[5,6]. Aspirin probably exerts its anti-defensive effect locally rather than on 1 central mechanism, its local action seeming to be a blockage of humoral mediators of defensive reactions, an interference with some step in a sequence in which the mediator, which may sometimes be a kinin or a slow-reacting substance, is involved. Piper and Vance[6] have shown that such a sequence includes the release of an unidentified smooth muscle contracting substance and that this release is blocked by aspirin[6].

It has been demonstrated by investigations with acetylsalicytic acid (aspirin), and other analgesics, that the analgesic effect of these drugs involves a peripheral component of action due to interference with the function of pain producing substances[8]. Distinct actions on peripheral nerves of analgesics with and without anti-inflammatory properties have been reported by Schorderet[9]. He found out that salicylic acid of 2 mM (0.36 mg/ml) blocked the conduction of excitation, increased the content of inorganic phosphate and decreased potassium concentration. Hartman supposes that nonnarcotic analgesics, amongst which he numbers acetanilide, phenacetin and phenzone, behave in a distinct manner as analgesics with anti-inflammatory properties.

On the other hand vitamin C (ascorbic acid) has been reported to be a cofactor in certain specific enzymatic reactions and is needed for all normal function. Some species are unable to synthesize vitamin E and must obtain it from exogenous sources. The function of vitamin E compounds (tocopherols) is not known with certainty but there is some good evidence that they are antioxidants, preventing the destructive nonenzymatic attack of molecular oxygen on the double bonds of the polyunsaturated fatty acid components of tissue lipids.

*Material and methods.* The rheumatoid and nonrheumatoid synovial cell line was obtained with the courtesy of Dr D. Ford, Director of Canadian Arthritis and Rheumatism Society, B.C. The normal rat muscle cells and the bone cells were obtained from 1-day-old Western Albino rats. After using ether for anesthesia, under sterile conditions pieces of the muscle were removed from 4 rats, placed in a petri dish containing Hank balanced salt solution (BSS) plus antibiotics. Pieces of the femur after removal of the tissue surrounding it were also transferred to a petri dish with BSS, mincing the muscle pieces as well as the bone with scissors to small fragments, washing them several times in BSS, then culturing the tissues with standard cell culture procedure for muscle[10]. After trypsinizing the tissue, the cells were counted using a hemocytometer and trypan blue for viability, with $10^4$–$10^5$ cells/flask inoculated in Falcon flasks using Eagle modified media supplemented with 10% calf serum and antibiotics (penicillin, streptomycin and fungazone; Grand Island Biological Company). Some explant cultures were used, but no experiment used a primary culture, but only after reinoculation. A method for bone culture[11] previously reported was utilized, using the same media. The cells were maintained in a 37 °C incubator, replacing the flask atmosphere with 5% $CO_2$ + air. When the cells reached confluency standard trypsinizing procedures were used to remove them from the flask surface where they grew as monolayers. The cells were counted and reinoculated for different experiments.

Aspirin solution. 2 types of solution were used, with low and high aspirin concentration. The high concentration was 0.18 g/100 ml ($10^{-2}$ M solution), the low 0.018 g/100 ml. More diluted concentrations down to $10^{-7}$ M have been utilized.

Vitamin C. Ascorbic acid was used with concentrations of 1 g/ml and 0.1 g/ml.

Vitamin E. Vitamin E was used in the succinate form with 1000 IU/100 ml. Since it is lipophilic, it does not dissolve in

water. It was dispersed by sonication, so the actual concentration was not known, but an effort was made to ensure homogenity before using any solution, and to ensure constant concentrations. High vitamin E means approximately 10 IU/ml.

Each experiment was started by inoculating the same number of cells into each flask, and applying the drug at the same time of inoculation. After a period of 1 week, the cells were trypsinized and counted. Aspirin was added every other day (assuming it was cleared fast), vitamin C and E were applied only once, and a change of media took place once a week. Bicarbonate was added as necessary to maintain correct pH.

*Results and discussions.* Aspirin has been consumed throughout the world in huge quantities, with over 4000 references cited for work on aspirin and similar compounds. Several different hypothesis have been advanced to explain the action of aspirin-like drugs. This includes an interference with oxidative phosphorylation[12], the displacement of an endogenous anti-inflammatory peptide from plasma protein[13-15], interference with the migration of leucocytes[16,17], inhibition of leucocytic phagocytosis[18], stabilization of lysosomal membranes[19], inhibition of the generation of lipoperoxides[20], and hyperpolarization of neuronal membranes[21-23]. Recently, interest in this field was stimulated enormously by the discovery[24-26] that aspirin-like drugs inhibit the synthesis of prostaglandins.

Table 1 described the effect of different aspirin concentrations on the arthritic rheumatoid (AR) synovial cells. In a study of the effect of aspirin on livers of AR and normal patients the serum level was raised to 25-30 mg/ml (in vitro), and at this level it was concluded that salicylate is mildly hepatotoxic in some persons, and this hepatoxicity may be augmented by systemic lupus erythematosis and other illnesses. Aspirin has been reported to cause abnormalities of liver function in patients with rheumatoid fever[27].

Lower dosages of aspirin were used but no effect could be detected; the range of dilution used was from $1\times10^{-2}$ to $1\times10^{-7}$ ml of a solution of concentration 1.8 mg/ml. Higher dosages as shown in table 1 produced a significant effect in inhibiting the growth of such cells.

There is a similar effect on the normal synovial cells and AR cells as shown in table 2.

It has been reported that salicylate produced experimental inhibition of bone formation in in vitro studies (organ culture), never in bone cells[28]. It has been found that salicylate depresses the biosynthesis of mucopolysaccharide sulfates and collagen in cartilage and bone. Futami reported the effect of aspirin in inhibiting bone growth in vivo[29]. He attributed this to the inhibition of the obstructive mechanism of the glycosaminoglycans by the action of sodium salicylate, if the interpretation that normal bone growth was inhibited by salicylate were accepted. Serum calcium and phosphate levels were not significantly different than control group, that means that no active cancerous bone formation was in progress. Also there is work reported by Daniel[30] that sodium salicylate at $10^{-3}$ M concentrations was able to limit the number of plaques induced by RNA-virus on monkey kidney cell cultures.

Effect of vitamin C and with aspirin combination is shown in table 4. Vitamin C did not affect the normal synovial cells, and it did not affect the population growth of nonrheumatoid cells, even with such high concentration. Since ascorbic acid will change the ph of the media, 7% $NaHCO_3$ was added to adjust the ph to be 7. Stone[31], summarizing more than 40 years of work on vitamin C, and Benade[32], publish reports describing their findings that ascorbate would kill cancer cells and was harmless to normal cells[32]. This finding is supported also by the study of ascorbate on Ehrlich ascites carcinoma cells in vitro, which is found to be highly toxic. It was suggested that vitamin C is a potential drug for anticancer use.

It has been found in the present study that ascorbate was lethal to AR synovial cells, suggesting potential as an anti-inflammatory drug. Table 5 summarizes such effect combined with aspirin, as aspirin has been given for pain relief from such inflammatory disease symptoms.

It can be seen from tables 4 and 5 that vitamin C eradicated the AR synovial cells, but it did not affect the normal cells. The explanation for such action is the hypothesis that vitamin C can act as an antioxidant as well as oxidant, by generating superoxide, assuming that there is a deficiency in the AR-cells of the enzyme superoxide dismutase, which protects the cell from peroxides and free radicals. This also suggests that normal cells have such an enzyme to catalyse

Table 1. Effect of aspirin on AR synovial cells

| Aspirin concentration (1.8 mg/ml) | Cell population $\times 10^4$ | Relative growth (%) |
|---|---|---|
| Control (0 aspirin) | 3.3 ±0.4058 | 100 |
| 0.1 ml | 0.88±0.1395 | 27 |
| 0.5 ml | 0.65±0.08 | 20 |
| 1 ml | 0.76±0.118 | 23 |

Table 2. Effect of high aspirin concentration on nonrheumatoid synovial cells

| Aspirin concentration (1.8 mg/ml) | Cell population $\times 10^4$ | Relative growth (%) |
|---|---|---|
| Control (0 aspirin) | 1.2 ±0.110 | 100 |
| 1 ml aspirin | 0.25±0.08 | 21 |

Table 3. Effect of aspirin on rat bone and muscle in vitro

| Aspirin concentration (1.8 mg/ml) | Cell population $\times 10^4$ | Relative growth (%) |
|---|---|---|
| Rat muscle cells | | |
| Control (0 aspirin) | 1.8 ±0.041 | 100 |
| 1 ml | 0.6 ±0.023 | 30 |
| Rat bone cells | | |
| Control (0 aspirin) | 14 ±0.2129 | 100 |
| 0.7 ml | 2.35±0.095 | 16.79 |
| 0.5 ml | 1 ±0.0447 | 7.14 |

Table 4. Effect of vitamin C and aspirin on cell population of nonrheumatoid synovial cells

| Treated groups | Cell population $\times 10^4$ | Relative growth (%) |
|---|---|---|
| No aspirin (control) | 7.5 ±0.204 | 100 |
| High aspirin | 0.25±0.08 | 21 |
| High vitamin C | 7 ±0.1915 | 93 |
| Low vitamin C | 6.9 ±0.18 | 92 |
| High aspirin + high vitamin C | 3 ±0.1 | 40 |
| Low aspirin + low vitamin C | 6.3 ±0.1202 | 84 |

Table 5. Effect of vitamin C and aspirin on AR synovial cells

| Group treatment | Cell population $\times 10^4$ | Relative growth (%) |
|---|---|---|
| No vitamin C, no aspirin (control) | 2.26±0.21 | 100 |
| High aspirin | 1.23±0.239 | 54 |
| Low aspirin | 1.4 ±0.282 | 62 |
| High vitamin C | 0 ±0 | 0 |
| Low vitamin C | 1.9 ±0.085 | 84 |
| High aspirin + high vitamin C | 0 ±0 | 0 |
| Low aspirin + low vitamin C | 1.2 ±0.239 | 53 |
| High aspirin + low vitamin C | 0.93±0.1333 | 24 |
| Low aspirin + high vitamin C | 0 ±0 | 0 |

$O_2^- + O_2^- + 3H^+ \rightarrow H_2O_2 + O_2$ protecting the cell from peroxides and free radicals[33]. An assay for such enzymes in a continuation to this work will give more evidence to such a hypothesis. Note that the aspirin dose is not added every other day as mentioned in earlier experiments but only added with vitamin C once.

The effect of aspirin and vitamin C on rat bone marrow cells and muscle cells is hard to relate to the human cells, as tolerance levels of vitamin C are different, and humans cannot synthetize vitamin C but some other mammals can. Table 6 shows the effect of vitamin C and aspirin on rat cells. This table indicates that high level of vitamin C was toxic but that it did not kill all the cells. It is likely that if the cells had been washed, and new media free of vitamin C were added, the cells would survive, unless their number got so low to the extent that contact is difficult and therefore they will die. Also, as mentioned before, the metabolic and growth rate of human cells are different, which suggests that it takes more effort to simulate the results from animal experiments in order to deduce results for the human.

The last experiment was conducted to test if vitamin E, which is an antioxidant, would possibly retard or stop vitamin C. Table 7 shows that the effect of vitamin E on the cells were very different, the cells did not die as fast as if vitamin C only applied. The morphology of the cells became very different, and the cells with the high vitamin C and E combination died after 2 weeks. Counting the cell number was difficult as the cell morphology changed and it was hard to say if a cell was viable, since it was not dyed by the trypan blue but showed an unclear border. This suggests that there was damage to the cells, and the vitamin E presence prolonged the cell survival for awhile, but ultimately the cells died.

*Conclusions.* It is found that aspirin markedly decreased the percent relative growth of both AR synovial and normal synovial cells, also normal rat muscle cells. The decrease in percent relative growth was about the same in all cases, a reduction to $\frac{1}{5}$ of the control, for concentrations of aspirin from 0.1 ml to 1 ml of 1.8 mg/ml solution. The reduction in percent relative growth for rat bone cells was even larger.

High and low vitamin C concentrations had little effect on normal synovial cells, and a low concentration had little effect on AR synovial cells. However, a high concentration of vitamin C eradicated these cells.

Table 6. Effect of aspirin and vitamin C on bone and muscle cells of rat

| Concentration of vitamin C and aspirin | Cell population $\times 10^4$ | Relative growth (%) |
|---|---|---|
| 0 (control) | 1.566 ± 0.314 | 100 |
| High vitamin C | 0.2 ± 0.0516 | 14 |
| High aspirin + high vitamin C | 0.2 ± 0.103 | 14 |
| Low aspirin + low vitamin C | 0.73 ± 0.116 | 52 |
| 0 (control) | 1.1 ± 0.152 | 100 |
| Low aspirin | 0.36 ± 0.06 | 33 |
| Low aspirin + low vitamin C | 0.36 ± 0.12 | 33 |
| High aspirin + low vitamin C | 0.3 ± 0.1612 | 27 |
| Low aspirin + high vitamin C | 0.02 ± 0.033 | 1.8 |
| High aspirin + high vitamin C | 0.03 ± 0 | 2.7 |

Table 7. Effect of vitamin C and E on AR cells

| Group treatment | Cell population $\times 10^4$ | Relative growth (%) |
|---|---|---|
| Control | 1.76 ± 0.158 | 100 |
| Low vitamin C + low vitamin E | 0.733 ± 0.183 | 42 |
| High vitamin C + high vitamin E | 0 after 2 weeks | 0 |

When aspirin was used in combination with vitamin C, vitamin C was far more important than aspirin in eradicating AR synovial cells. High aspirin and high vitamin C eradicated AR synovial cells, but also significantly reduced normal synovial cell percent relative growth. The most effective combination for reducing AR synovial cell growth, while having a small effect on normal synovial cells, appears to be low aspirin plus low vitamin C.

The pattern of effects of vitamin C and aspirin on rat bone and muscle cells is similar, in that high vitamin C drastically reduces percent relative growth, with or without aspirin. The most effective combination appears to be high vitamin C with high aspirin, the least low vitamin C and low aspirin.

Indications were obtained that vitamin E, acting on AR synovial cells, retards the cell eradication effect of vitamin C but does not prevent it. The presence of vitamin E induced morphological changes in the cells not seen with vitamin C. Further studies are needed here to establish any possible anti-oxidant effects of vitamin E on vitamin C action.

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