Although sulfaguanidine and iodide are known to act synergistically on thyroid(7), addition of 2% sulfaguanidine (SG) to these high-iodide diets appears to slow the evolution of the inflammatory response in submaxillary glands. Animals receiving 4% NaI and SG reveal the combined acute and chronic glandular inflammatory response described, while those receiving 4% NaI alone show only the more chronic picture. With 0.5% NaI, animals also receiving SG show a less intense periductal inflammation and a more limited ductal epithelial proliferation, with frequent intact duct-metaplastic duct junctions.

Notwithstanding the distal inflammatory change already described, the glandular unit (acini, granular tubules and intralobular striated ducts) was intact in all groups. This suggests several possible mechanisms of action of massive doses of iodide: 1. a distally progressive concentration gradient of iodide may be produced in saliva, 2. the distal duct-structures may be more sensitive than proximal glandular areas to iodides circulating or in the saliva, 3. the effect may be mediated through some other organ, 4. the inflammatory response may be to some infectious agent activated by high iodide levels, 5. massive iodide doses in rats may produce distal salivary duct iodide concentrations obtained in other species with smaller doses. This study does not identify which, if any, of these processes is operative.

Summary. Submaxillary gland sialadenitis has been produced in rats on diets containing 0.5% and 4% NaI. This inflammatory and metaplastic process selectively involves distal ductular structures and is modified by concomitant administration of 2% sulfaguanidine.

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Inhibition of T'ang Trachoma Agent by 5'-Fluorodeoxycytidine.* (28671)

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The incorporation of halogenated pyrimidines into bacterial cells was described by Dunn and Smith(1). Heidelberger *et al.*(2) reported on the anti-tumor activity of the fluorinated pyrimidines. The inhibitory effect of these drugs was studied extensively on human cell lines(3) and on neoplastic diseases in animals(4) and men(5).

Fluorinated pyrimidines interfere with DNA synthesis by inhibition of thymidylate synthetase in tumor cell systems(6) or creation of thymine deficiency in bacterial cells (7). Production of viral DNA was stopped by addition of the drugs to infected cultures as observed by plaque inhibition(8), by interference with virus propagation(9), and by alteration in function of virus infected cells (10). Addition of the fluorinated pyrimidines to cultures at intervals after virus infection offers a method by which the time course of viral DNA synthesis can be followed. Tanami *et al.*(11) studied the effect of FU and FUDR on the agent of psittacosis at various stages of the growth cycle and reported inhibition in DNA synthesis which could be reversed by uracil and thymidine. Green(12) described the time correlation be-

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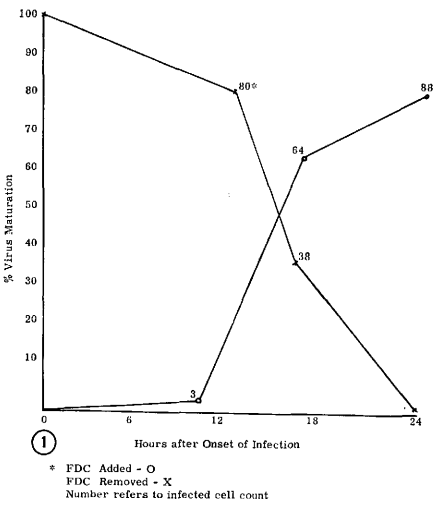

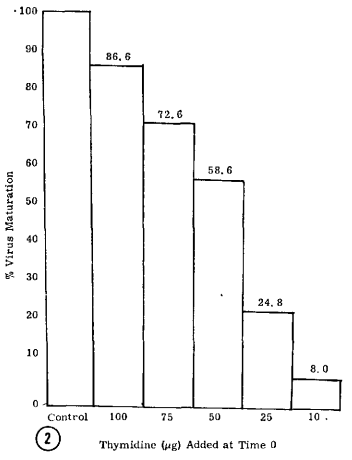

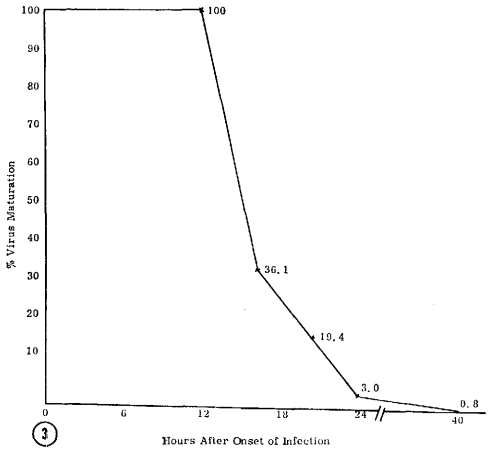

FIG. 1. Sensitivity of T'ang trachoma agent to 5'-fluorodeoxycytidine.

FIG. 2. Correlation between thymidine concentration and maturation of T'ang trachoma agent in cultures containing 10 μg 5'fluorodeoxycytidine.

FIG. 3. Reversal effect of thymidine added at intervals of 5'fluorodeoxycytidine T'ang infected cultures.

tween adenovirus DNA synthesis and drug addition; and he assumed that 7-21 hours was the most active stage in viral DNA production.

The agent of trachoma contains DNA and has a replication cycle of approximately 48 hours. This agent was used with 5'fluoro-2'deoxycytidine to clarify the time course of DNA synthesis.

Materials and methods. The experiments involved McCoy cells and T'ang strain of trachoma virus as described previously(13, 14). The virus and 5'fluoro-2'deoxycytidine (5 FDC, received from Dr. Robert Duschinsky, Hoffmann-LaRoche Co.) were diluted in nutrient fluid (NF) which consisted of mixture 199 with 0.5% lactalbumin hydrolysate, 5% heat inactivated calf serum and 100 μg streptomycin sulfate per ml. The assay for infectious virus particles was carried out by a technique involving acridine orange staining and fluorescence microscopy(15).

Results and discussion. Treatment of infected and normal cultures at time 0 with graded amounts of 5-FDC showed a high therapeutic index. The normal cells were not altered by cultivation in NF containing 400 μg of drug per ml and 2.5 μg/ml stopped the growth of 100% of the virus. It was clear that the effect of 5-FDC was on the virus and was not obviously mediated through damaged cells. The long growth cycle (48 hours) of this virus provided a useful system for determination of the stage or stages of sensitivity of the virus to the drug. 5-FDC was added to infected cells at time 0. After onset of infection the drug supplemented medium was replaced with fresh N.F. Other tubes were inoculated with virus in N.F. and 5-FDC was added to cultures at intervals thereafter.

As summarized in Fig. 1: treatment for 12 hours and for 16 hours inhibited 20% of the virus (*i.e.* 80% reached maturity) and 62% (*i.e.* 38% reached maturity) respectively. Treatment for 24 hours caused 99% inhibition of the virus. A similar sensitive stage at

TABLE I. Reversal of 5'-fluorodeoxycytidine Effect on T'ang Trachoma Agent.

Compound (100 μg)*	% virus maturation
Thymidine	86.6–100
Uridine	3.6– 6.0
Uracil	1.0– 2.8
Uridine monophosphate	0
Cytosine	0
Deoxycytosine	1.0– 2.7
Deoxycytidylic acid	0

* Added at time 0 to N.F. containing 10 μg 5-FDC.

12-16 hours after onset of infection was found in the second set of experiments. Maturation of the virus was inhibited if 5-FDC was added directly after inoculation of the cultures or at 10 hours later. If the drug was added at 16 hours after onset of infection, 64% of the virus particles reached maturity. Treatment at 24 hours after onset of replication caused less inhibition.

The sensitivity of replicating trachoma agent for 5-FDC was distinct at 12-16 hours after onset of infection. The fluorinated pyrimidines interfere with DNA synthesis(6). The above data suggested that the greatest synthesis of new DNA in the trachoma agent takes place at this 12-16 hour stage of replication.

Deoxycytidine may be incorporated into DNA by either 1) deamination and methylation then incorporation through thymidine, 2) deamination and incorporation through deoxyuridine, or 3) incorporation of cytosine base directly to DNA. 5-FDC may block DNA synthesis in one or more of the above channels. The next step was to find those compounds, if any, which would reverse the inhibitory effect of 5-FDC. Thymidine, uridine, uridine monophosphate, uracil, cytosine, deoxycytidine and deoxycytidylic acid were tested. At time 0 a mixture containing 100 μg of each compound, 5-FDC (10 μg) and virus were added to McCoy cells. Thymidine reversed inhibitory effect of 5-FDC on virus; however, there was partial reversal or none with the other compounds (Table I). Graded concentration of thymidine with 10 μg 5-FDC were added to infected cultures and the amount of virus growth was assayed. A correlation could be demonstrated between the concentration of thymidine and virus maturation: 100 μg thymidine—86.6%, 75 μg—72.6%, etc. (Fig. 2). Since 100 μg thymidine at time 0 enabled most of the virus to attain maturity in the presence of 5-FDC, we determined how late after onset of infection 100 μg thymidine would reverse the inhibitory effect. Virus and 5-FDC were added to cell cultures and at intervals thereafter 100 μg of thymidine were added. Addition of thymidine to the cultures infected between 0-12 hours resulted in 100% replication of virus. Thereafter, the reversal effect of thymidine declined sharply (Fig. 3).

In the cell-virus system used herein most of the trachoma DNA synthesis occurred between 12-16 hours after onset of replication. This represented the sensitive stage of the reaction to the drug. Addition of thymidine before this sensitive stage competed with the 5-FDC and mature virus was formed; however, when thymidine was added later, the effect of 5-FDC was not reversed. The small amount of virus which completed its maturation cycle may be of the slow growing type.

Summary. Maturation of T'ang trachoma agent in McCoy cells was inhibited with high therapeutic efficiency by 5-FDC. The virus was most sensitive to 5-FDC at 12-16 hours after onset of infection which may be the period in which new viral DNA was synthesized. The inhibition by 10 μg of 5-FDC was reversed by 100 μg of thymidine at 0-10 hours after infection, and the reversal effect declined thereafter.

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Bone Cellular Activity Associated with Skeletal Atrophy from Disuse.* (28672)

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Disuse of the skeletal system results in its atrophy with development of osteoporosis. Mechanical stresses and strains seem essential for normal bone formation. If these are absent, osteoblastic activity is reduced while resorptive processes continue at a normal pace resulting in a net loss of bone. However, little is known concerning the specific metabolic behavior of bone cells during osteoporosis of disuse.

Investigations with immobilized subjects generally show that the degree of atrophy is proportional to the extent of non-use. Such studies(1,2,3) have been based on urinary excretion of calcium and phosphorus, loss of bone ash weight or a decrease in bone breaking strength after selected periods of immobilization. In the current study the metabolic activity of bone cells during bone loss resulting from disuse was determined.

Methods. Young male rats of the Sprague-Dawley strain weighing 300-400 g were anesthetized with nembutal and the right rear leg immobilized with a plaster of paris cast. After one or 2 weeks of immobilization the rats were sacrificed and the tibia from each leg was removed, cleaned and weighed. The metaphyseal area of this bone and that from the distal end of the femur were split and washed in iced Krebs-Ringer bicarbonate medium, blotted dry with filter paper and weighed on a Roller-Smith balance. The samples were then placed in incubating flasks containing iced medium and stored on ice until the start of incubation. Similar samples were saved for cell nitrogen and citric acid determinations.

All samples were incubated in Warburg flasks at a temperature of 37°C for 3 hours. Approximately 100 mg of wet bone were used in each flask. Calcium-free Krebs-Ringer bicarbonate media buffered to a pH 7.4 was used. The 3 ml sample also contained 2 mg of glucose per ml as the substrate and 5 U of penicillin and 0.01 mg per ml of streptomycin were added to prevent bacterial growth. The solution was gassed with a 5% CO_2-95% O_2 mixture for 10 minutes before the bone sample was added and just prior to incubation the flask was gassed with the same mixture.

Following incubation, aliquots of the media were analyzed for citric acid by a modification of Natelson *et al.*(4), glucose by the method of Huggett and Nixon(5) and lactate by the Barker and Summerson(6) technique. Bone cellular content was estimated by determining the non-collagenous nitrogen level employing a modification of Lilienthal *et al.* method(7) as described by Borle *et al.* (8).

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