sulted from a direct effect of $T^3$ on bone. However, other investigators(17,18) have observed this type of bone lesion in rats fed calcium-deficient diets. It is therefore possible that excess thyroid hormone has both a) a direct effect on bone, promoting resorption, and b) an indirect effect whereby decreased intestinal calcium absorption results in insufficient calcium to maintain proper mineralization of bone.

*Summary and conclusions.* Intestinal active transport of calcium was studied by an *in vitro* gut sac technic in rats with induced hyperthyroidism. An excess of thyroid hormone resulted in a prompt and persistent decrease in calcium transport, and also in a decrease of trabecular bone. The mechanism of this action of thyroid hormone and its physiologic significance is yet to be determined. It is suggested that decreased calcium absorption may be a significant factor in the negative calcium balance and bone changes found in some hyperthyroid patients.

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## Thymidine Kinase in IDU Resistance.* (30433)

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It has been established that serial passages of herpes simplex viruses in increasing concentrations of 5-iodo-2′-deoxyuridine (IDU) can result in the development of mutant viruses which are resistant to IDU as well as bromodeoxyuridine (BDU)(1-3). The action of both drugs is dependent on their phosphorylation and incorporation into DNA: Cells which are deficient in thymidine kinase (TK—) are resistant to BDU(4) and can also withstand high concentrations of IDU which are lethal to normal LM cells(5).

Herpes simplex virus induces infected cells to produce large amounts of thymidine kinase and it has been postulated that virus resistance to IDU may be caused by the loss by the virus of the ability to induce thymidine kinase synthesis in its host. The extent to which the virus strains are inhibited by IDU

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and BDU might depend, however, not only on the genetic ability of the virus to induce thymidine kinase, but also on the normal host cell thymidine kinase content, since normal cells can catalyze the phosphorylation of these drugs(6).

The purpose of this study is to ascertain whether the observed IDU resistance of herpes simplex *in vivo* is due to a lack of thymidine kinase activity.

*Methods and materials. Cell lines.* Mouse fibroblast L-929 purchased from Microbiological Associates and a strain with a reduced level of thymidine kinase LM (TK—) kindly donated by Dr. Ghosh were used in this study. In order to be certain of the initial levels of thymidine kinase, the cell lines LM (TK—) and normal L-929 were tested for their ability to incorporate $H^3$ thymidine. It was found that the incorporation by normal L-cells was at least 4 times as great as the incorporation into the thymidine kinase-deficient L-cells. Monolayer cultures were grown in 4 oz. bottles with Eagle's Minimum Essential medium supplemented with 10% calf serum and 1% L-glutamine. The bottles were seeded with $2 \times 10^5$ cells per ml, incubated at 37°C and used the second day after subculture. Suspension cultures were propagated in screw cap tubes with similar medium, but deficient in calcium. The cells were pooled and dispensed in 20 ml aliquots at concentration of $2 \times 10^5$ cells per ml just prior to use.

*Virus strains.* Normal herpes simplex virus ATCC HF378, an IDU resistant virus strain (IDU-R)(3) and, mutant 2006 which is unable to induce thymidine kinase synthesis(5), were studied. Virus stocks were prepared in 32 oz. stationary cultures of LM (TK—) cells. After evident CPE, the cells were scraped off, disrupted by ultrasound, and stored in sealed glass vials at —90°C. Titration of the virus stock was done with 5 cultures per 10-fold dilution of the virus and end points were calculated by the method of Reed and Muench(7). The IDU-R virus was passed in LM (TK—) cells and after passage it remained resistant to IDU *in vivo*.

*Enzyme studies.* Incorporation of $H^3$ thymidine was studied as an index of thymidine kinase activity in both monolayers and suspension cultures. Six hours after infection, radioactive thymidine (10 microcuries) was added to the cultures and incubated for 30 minutes at 37°C. The cells were chilled and spun down. After 2 washes with 5% TCA in the cold, the nucleic acid were hydrolyzed with 0.5 M perchloric acid at 70°C for 20 minutes. The extract was analyzed for DNA by the procedure of Burton(8). The radioactivity of the hydrolysate was measured with a liquid scintillation spectrometer for a 10-minute period. Other thymidine nucleotides were analyzed by paper chromatography. One hundred microliters of the TCA supernate was spotted in Whatman No. 4 paper with 20 μg each of carrier TMP, TDP and TTP. The nucleotides were resolved by descending chromatography using iso-butyric-$NH_4OH$ and water as the solvent(9). Paper blanks and the nucleotide spots were cut out, eluted with 0.1 ml of 0.1 N HCl for 1 hour at room temperature and counted as previously described.

*In vivo* studies of herpes simplex keratitis were done as previously described(10). Virus was inoculated in the rabbit cornea; 2 days after infection the eyes were examined and distributed into comparable groups of 12, and Stoxil,† a commercial 0.5% IDU ointment, was applied every 2 hours. Final evaluation of the ulcers was done on a double-blind basis on the third day of treatment. The scoring was done as follows:

1—ulcers covering ¼ of the cornea
2—ulcers covering ½ of the cornea
3—ulcers covering ¾ of the cornea
4—ulcers covering the entire cornea

*Results.* The ability of the virus strains to induce thymidine kinase was assessed by studying the incorporation of $H^3$ thymidine by mouse fibroblasts in infected and non-infected cultures. The results (Table I) showed that LM (TK—) cultures when infected with the thymidine kinase-deficient mutant 2006 failed to incorporate $H^3$ thymidine above the level of non-infected controls, while those infected with the IDU-R strain showed a high level of incorporation which was similar to those infected with the IDU sensitive strain,

† Smith, Kline & French, Philadelphia, Pa.

TABLE I. Incorporation of $H^3$ Thymidine by Mouse Fibroblasts Infected with Herpes Simplex.

| Exp | Cell strain | Virus strain | cpm/$\mu$g DNA |
|---|---|---|---|
| a | LM (TK–) | HF378 | 115 |
| | | 2006 | 54 |
| | | IDU-R | 160 |
| | | None | 60 |
| b | L-929 | HF378 | 211 |
| | | 2006 | 168 |
| | | IDU-R | 211 |
| | | None | 233 |

TABLE II. Distribution of Radioactivity Within the Thymidine Nucleotides.

| Cell line | Virus strain | cpm | | |
|---|---|---|---|---|
| | | TTP | TDP | TMP |
| L-929 | — | 11 | 129 | 593 |
| | 2006 | 11 | 58 | 276 |
| | IDU-R | 185 | 343 | 1023 |

HF378. These findings show that IDU-R and HF378 viruses have the capacity to induce thymidine kinase activity in LM (TK—) cells. In normal L-929 cells, thymidine incorporation of HF378 and IDU-R infected cultures was comparable to that of the non-infected control, while the cultures infected with mutant 2006 exhibited a lower level of incorporation than the controls.

Since incorporation of TdR into DNA requires the phosphorylation of TdR to TMP, TDP and TTP, the nucleotide pool of infected cultures was studied (Table II). More labeled TMP, TDP and TTP was found in cultures infected with IDU-R than in those infected with mutant 2006. It is evident from these results that thymidine kinase activity is not only present but enhanced in IDU-R infected cultures.

The effect of IDU on growth of herpes simplex *in vivo* was also investigated (Table III). The thymidine kinase-deficient strain, 2006, as well as the normal HF378 were sensitive to IDU, while the IDU-R strain was completely resistant to IDU.

TABLE III. Effect of IDU on Growth of Herpes Simplex Virus *in vivo*.

| Virus strain | Ulcer grade | |
|---|---|---|
| | Treated | Control |
| IDU-R | 2.25 ± .39 | 2.91 ± .29 |
| 2006 | .82 ± .48 | 2.65 ± .61 |
| HF378 | .10 ± .13 | 2.16 ± .47 |

*Discussion.* It has been shown by Kit(11) that thymidine kinase activity is induced in L-cells after infection with herpes simplex and that the synthesis of several enzymes associated with DNA synthesis appears to be regulated by the viral genome.

In our experiments with infected mouse fibroblasts, a) infection with mutant 2006 did not increase thymidine kinase activity above the cell control in LM (TK—) cells and decreased such activity in L-929 cells; b) HF378 and IDU resistant strains enhanced thymidine kinase activity above the cell control in LM (TK—) cells. Such findings indicate that thymidine kinase inducing activity was present in the IDU resistant virus as well as the normal HF378, whereas mutant 2006 was devoid of thymidine kinase inducing activity. The distribution of radioactivity within the thymidine nucleotides in infected cultures of L-929 confirms this, as more radioactivity was found in the nucleotides of IDU-R infected cultures than in those infected with mutant 2006. IDU resistant virus causes appreciably more thymidine kinase induction than the mutant 2006.

In the *in vivo* studies of herpes simplex keratitis produced by these viruses, the difference between both strains is even more marked. The mutant 2006 is normally sensitive to IDU while IDU-R strain is completely resistant to IDU. The IDU-R strain is sensitive to $F_3$TdR, however, although $F_3$TdR, like IDU, must be phosphorylated to be active(3). IDU sensitivity of mutant 2006 may well be due to the phosphorylation of IDU by the host cell enzymes, but that resistance to IDU in our IDU-R strain must be due to a mechanism other than lack of thymidine kinase. Our experiments show that thymidine kinase inducing activity is present in virus resistant *in vivo* to IDU and that virus deficient in the ability to induce thymidine kinase remains sensitive to IDU *in vivo*.

*Summary.* Studies were done to determine whether 5-iodo-2′-deoxyuridine resistance of herpes simplex in rabbit corneal infection was a function of thymidine kinase deficiency in the viral genome. Herpes simplex virus passed in rabbit kidney cells in the presence

of 5-iodo-2′-deoxyuridine was resistant to it *in vivo* although this resistant virus induced normal amounts of thymidine kinase in mutant LM cells deficient in thymidine kinase. A different strain deficient in thymidine kinase was susceptible to 5-iodo-2′-deoxyuridine *in vivo*, presumably because 5-iodo-2′-deoxyuridine was phosphorylated by cellular thymidine kinase. Viral resistance to 5-iodo-2′-deoxyuridine, unlike the resistance of cancer cells, cannot depend solely on the loss of thymidine kinase inducing ability.

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## Sodium and Potassium Intake, Blood Pressure, and Pressor Response to Angiotensin.* (30434)

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In several forms of experimental hypertension the induced elevation in blood pressure is related to the amount of sodium(1-4) and potassium(5,6) in the diet. In addition, the intake of both these ions influences the rate of aldosterone secretion(7,8), while only sodium affects the granularity of the juxtaglomerular cells(9). The studies of Dahl and Love(10) suggest that increased consumption of salt may be an etiological factor in the development of essential hypertension. Moreover, it has been shown repeatedly that a low sodium diet can often lower the blood pressure of hypertensive patients(11,12). The work of Tobian(13,14) and others(15, 16) on experimental animals suggests an increase in intracellular potassium, sodium, and water occurs in the vascular smooth muscle of hypertensive animals. The exact mechanisms whereby sodium and potassium influence blood pressure are not known. One possibility might involve an alteration in vascular responsiveness to circulating pressor agents. The present paper describes the effects of varying the concentrations of dietary sodium and potassium on the pressor sensitivity to angiotensin in rats.

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*Methods.* Male Sherman rats weighing 90 to 130 g were divided into 8 groups of 8 rats each plus a control group of 16 rats. For approximately 8 weeks they were fed diets which differed only in the concentrations of sodium and potassium. The diets, prepared by General Biochemicals, Chagrin Falls, Ohio, were as follows:

| Diet | Sodium | Potassium |
|---|---|---|
| Control | .25% | .56% |
| High Na | 3.01 | .52 |
| Low Na | 0 | .65 |
| High K | .25 | 4.19 |
| Low K | .60 | 0 |
| Low Na - Low K | .025 | .004 |

In addition, 1% NaCl, 1% KCl, or 1%