$$Vd = \frac{Vf}{1 - a}$$

and is equal to 12.5 mg/day for the controls and 14.7 mg/day for the TCT-treated animals.

The rise in urinary calcium output under TCT treatment is not significant; this means that the kidney is not involved in the mechanism of TCT-induced hypocalcemia. This finding is consistent with the observation that nephrectomy does not prevent the hypocalcemic response to TCT(9,10).

Conclusion. Thyrocalcitonin is able to produce and maintain a low plasma calcium level in an intact animal fed a normal diet. The marked inhibition of bone catabolism by thyrocalcitonin would suggest that this is the main mechanism involved in the lowering of the plasma calcium. Since bone anabolism is also decreased (a factor which would tend to raise plasma calcium) any contribution to the lowering of plasma calcium from this process would appear unlikely. Thyrocalcitonin administration results in an increased calcium balance. The utilization of dietary calcium by the gut is enhanced by TCT. In summary, thyrocalcitonin is a *calcium-sparing hormone.*

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Inhibition of J-128 (Osgood) Cell Culture by Azaserine.* (31444)

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Azaserine interferes with several glutamine catalyzed enzyme reactions, and it has been concluded that the primary site of action involves inhibition of conversion of formylglycineamide-ribotide to formylglycineamidine-ribotide(1). This assumption has been made on the basis that amino-imidazole carboxamide (AICA) will reverse the inhibitory effect of azaserine on the growth of *E. coli* cells (2). This interpretation must be questioned, however, as several amino acids also reverse the inhibitory effect of the drug(2). In a preliminary report, we presented evidence that inhibition of the growth of an established cell line in tissue culture by azaserine was not reversed by addition of hypoxanthine, adenine or AICA to the medium(3). This cell line (Osgood J-128) utilized hypoxanthine, adenine or AICA in preference to formation of purine by *de novo* synthesis, thus it was apparent that the drug interfered with some biochemical reaction other than blockade of *de novo* purine synthesis. The data suggested that azaserine did not exert its action by blocking conversion of xanthylic to guanylic acid, a reaction which also has been demonstrated to be inhibited by azaserine *in vitro*(4). However, the evidence was not clear cut since the culture converted guanine to guanylic acid at

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TABLE I. Failure of Hypoxanthine, Adenine or AICA to Reverse the Inhibitory Effect of Azaserine on Growth of J-128. See text for details.

Additions to media* (μg/ml)	Cells day 4† / Cells day 0
None	4.0
2 μg azaserine	.8
Idem + 40 μg AICA	1.0
" + 40 μg hypoxanthine	.9
" + 40 μg adenine	.9
1 μg aminopterin	1.0
Idem + 40 μg hypoxanthine	4.2
" + 40 μg adenine	4.3
" + 40 μg guanine	.9
" + 40 μg guanosine	1.1

* Media supplemented with 40 μg/ml of thymidine. See text for other details.
† Values for one experiment. Identical results obtained in 20 duplicate experiments.

a slow rate. The data presented below suggest that azaserine interferes with the growth of J-128 in a manner quite analogous to that reported for the carcinostatic activity of certain mustards.

Methods. Culture methods have been described elsewhere(3). At the end of the experimental period, the media was decanted from the layers of cells attached to the glass bottles, and the cell layers washed several times with saline. Fifteen ml of 0.1 M citric acid were added to each of the culture bottles and the bottles left at 37.5°C for several hours. The cells were then ruptured by vigorous shaking and the number of nuclei determined by counting a small aliquot in a bright line phase hemocytometer chamber. The nuclei were harvested by centrifugation and the amount of DNA determined by the diphenylamine reaction. Cells were cultured in the presence of C^{14} thymidine for 4 days, and the nuclei processed by the method described above to verify that no significant loss of DNA occurred during the procedure.

Results and discussion. The failure of hypoxanthine, adenine or AICA to reverse the inhibitory effect of azaserine on growth of J-128 cells is apparent from the data in Table I. Reversal of the inhibitory effect of aminopterin by hypoxanthine or adenine substantiates isotope data presented elsewhere that this cell line utilizes these purines(3). As was expected, neither guanine nor guanosine reversed the inhibitory effect of aminopterin.

Fig. 1 compares the rate of DNA synthesis by cells cultured in the presence of AICA and AICA plus azaserine. Although the drug produced a lag in synthesis of DNA for the first 24 hours, by day 2 the amount of DNA per culture equaled that of the control, and on

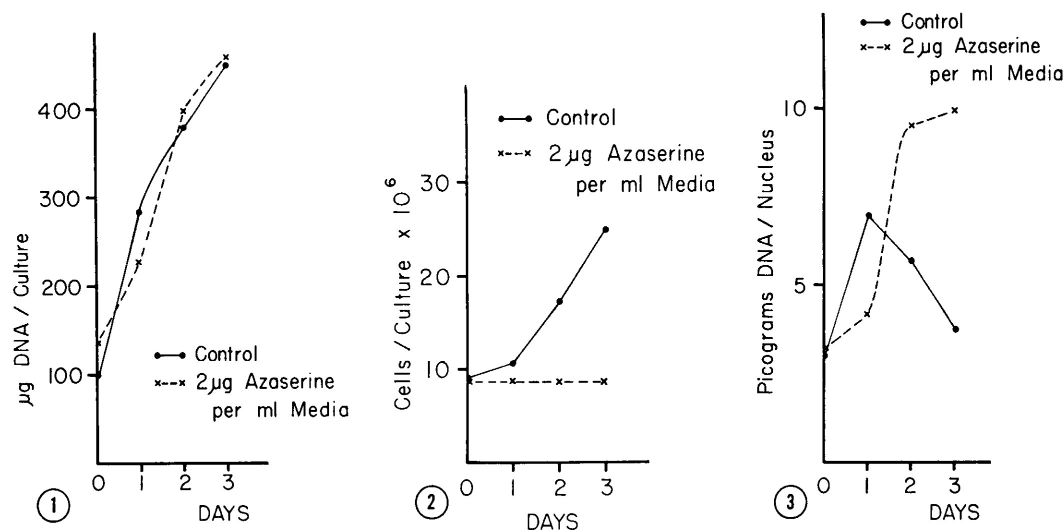

FIG. 1. Comparison of DNA synthesis in control J-128 cultures, and cultures exposed to azaserine. Culture media contained 40 μg of AICA/ml. Similar results obtained in 12 experiments.
FIG. 2. Comparison of cell growth of control cultures and cells cultured in media containing azaserine. All media contained 40 μg AICA/ml. Similar results obtained in 12 experiments.
FIG. 3. Comparison of DNA per nucleus of control cells and cells cultured in media containing azaserine. All media contained 40 μg AICA/ml. Similar results obtained in 12 experiments.

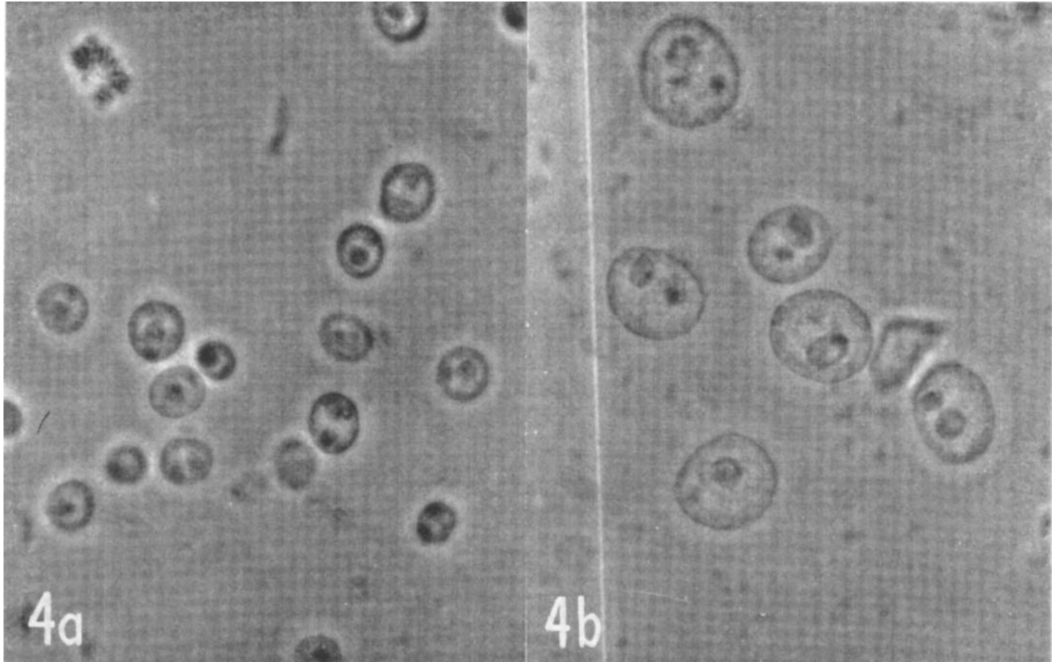

FIG. 4. Comparison of nuclei obtained from cells cultured in control media (a) and cells cultured in media containing azaerine (b). Direct phase contrast, original magnification 400 ×.

day 3 the amount of DNA per culture was identical to controls. It may be observed, however, in Fig. 2 that no cell growth occurred in the azaserine-treated cultures over the experimental 3-day period. When the amount of DNA per nucleus was plotted (Fig. 3) against days, it was again apparent that azaserine produced a lag in DNA synthesis during the first day. This lag was observed in 12 other experiments and in no experiment did the DNA content per nucleus double until day 2 or when azaserine was in the media. In 12 control experiments the DNA per nucleus doubled the first 24 hours. The DNA content per nucleus of azaserine-treated cells increased markedly for the next 24 hours, and, by day 3, the DNA content per nucleus was twice that of control nuclei. Thus, it would appear that the DNA of the azaserine-treated cells doubles and redoubles a second time, although cell division does not occur. The increase in the DNA content per nucleus of the azaserine-treated cells is paralleled by an increase of both cell size and nuclei size (Fig. 4). This increase in size of isolated nuclei is not an artifact since it can also be observed by phase contrast microscopy of living cells. Except for the increase in size, no marked difference was noted between the control nuclei and nuclei from cultures exposed to azaserine. However, lesions were present in the central sphere of attraction in many of the cells exposed to azaserine.

The data presented demonstrate that azaserine stops reproduction of J-128 cells by interfering with some reaction other than *de novo* purine synthesis. The possibility that azaserine also blocks *de novo* purine synthesis is not precluded by these data since all experiments were carried out in the presence of AICA. J-128 cells exposed to azaserine in medium not containing AICA markedly increase in size which would suggest that *de novo* purine synthesis is not seriously inhibited. However, it is very difficult to obtain a concentration of azaserine which will produce stasis over the 3-day period with only minimal cell death occurring. The impression is gained that the amount of azaserine which will cause stasis does not seriously impair *de novo* purine synthesis, but that a small increase probably causes a blockade of *de novo* purine synthesis, resulting in rapid cell death.

Summary. Adenine, hypoxanthine or AICA

will not reverse the inhibitory effect of azaserine on the reproduction of J-128 cells in tissue cultures. Cells exposed to azaserine increase markedly in size with a parallel increase of nuclei size and DNA content. The drug appears to act quite similarly to certain mustards by stopping cell division between S and G_2. A lesion appears in the central sphere of attraction in azaserine-treated cells, increases in size and apparently causes death of the cell.

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Effect of Diabetogenic Hormones on Transport of Glucose in Small Intestine *in vitro*. (31445)

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The intestinal absorption of glucose is diminished after surgical removal of the hypophysis(1) or the thyroid gland(2). In hyperthyroid patients the rise in blood sugar level in the glucose tolerance test is higher when glucose is fed than when it is injected (3). This indicates the facilitatory effect of the thyroid hormone on intestinal absorption of glucose. The absorption of glucose is greatly reduced in adrenalectomized animals(4). This observation, however, has been contradicted(2,5). Methods employed in the above studies have limitations. We, therefore, studied the effect of the diabetogenic hormones on absorption of glucose by the everted small intestinal sac technique of Wilson and Wiseman (6).

Materials and methods. Male albino rats weighing between 150 and 250 g were used. Different groups of rats were given treatments as follows:

Adrenocorticotrophic hormone (ACTH). One International Unit per 100 g body weight was injected intramuscularly daily for 10 days and the animal was sacrificed for the study of glucose absorption on the eleventh day.

Hydrocortisone and desoxycorticosterone. 2.5 mg hydrocortisone or 1 mg desoxycorticosterone was injected daily to each rat for 6 days and the animal was killed on the seventh day. *Thyroid gland.* 15 mg desiccated thyroid gland powder or 15 mg methyl thiouracil in a watery suspension was fed to each rat for 6 days and the animals killed on the seventh day.

All the animals were fasted for 24 hours before they were killed by stunning and decapitation. Everted sacs of the intestine were prepared(7), filled with Krebs-Ringer-bicarbonate saline with a 0.3% glucose concentration, incubated in a shaking incubator(8) and glucose in the serosal and muscosal fluids was estimated(9).

Results. Significantly increased transference of glucose was observed in the intestinal sacs of rats treated with ACTH, hydrocortisone or desoxycorticosterone. The transference did not change in the intestinal sacs of animals treated with thyroid or methyl thiouracil. The results are given in Table I.

Discussion. ACTH, hydrocortisone and desoxycorticosterone enhanced the intestinal transport of glucose. The effect might have been either a direct action of the hormones on the intestinal mucosal transport mechanism or their indirect effect *via* the adrenal cortex which is stimulated. In hypoadrenocorticism there is increased urinary excretion of sodium