to be established that a prolactin-inhibitory neuro-hormone is actually present in the CNS.

Summary. 1. Sixty mature female rats were injected with 10 μg estradiol daily for 10 days to develop their mammary glands. On days 1, 5 or 7, all except 10 control rats were each implanted underneath the kidney capsule with a pituitary from a similar untreated donor rat; on day 11, 26 implanted rats were hypophysectomized. All rats were killed on day 16. 2. Control mammary glands regressed to a bare duct system and showed no secretion, whereas 23 of 24 intact rats with pituitary grafts and 12 of 26 hypophysectomized rats with grafts showed mammary secretion and/or lobule-alveolar development. Most rats with pituitary implants had ovaries with large corpora lutea and few or no follicles. These results demonstrate that grafted pituitaries in the rat secrete greater than normal amounts of prolactin.

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Chemotherapy of Sarcoma 180 by Combinations of DL-Glyceraldehyde with 6-Thioguanine or with Azaserine and 6-Chloropurine.* (25802)

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Glyceraldehyde has been reported in a number of instances to be a potent glycolytic inhibitor; this was recently reviewed briefly by Gold(1). The mechanism of inhibition was shown by Lardy *et al.*(2) to be due to condensation of L-glyceraldehyde with dihydroxyacetone phosphate to yield L-sorbose-l-phosphate, which subsequently interfered with the hexokinase reaction. In view of this inhibition and of the knowledge that nucleotide formation requires carbohydrate as well as purine or pyrimidine moieties, the present study was designed to test anti-neoplastic properties of combinations of inhibitors of these systems.

Methods. Experiments were performed on 9 to 11-week-old female Ha/ICR Swiss mice (A. R. Schmidt Co.). Tumor transplantation was carried out by withdrawing ascites fluid from a donor mouse bearing a 7-day tumor growth. The fluid was centrifuged for 2 minutes in a clinical centrifuge (1600 x g), supernatant peritoneal fluid was decanted, cells were diluted 1:10 with isotonic saline, and 0.1 ml (approx. 2 x 10^6 ascites cells) of cell suspension was inoculated into each animal. Mice were distributed into groups of comparable weight and maintained during experi-

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TABLE I. Effects of DL-Glyceraldehyde and 6-Thioguanine on Survival Time of Sarcoma 180 Tumor-Bearing Mice.

Daily dose (mg/kg)		Avg survival (days)	No. 50-day survivors*	Avg wt (g)†
Glyceraldehyde	Thioguanine			
0	.0	11.8	0/34	+4.4
0	1.0	20.5	0/25	+4.8
0	2.0	19.5	0/10	+4.0
0	3.0	12.5	"	+2.7
200	.0	19.9	"	+1.5
400	.0	26.6	3/25	− .2
600	.0	29.6	3/20	+ .1
800	.0	18.7	0/10	− .4
200	1.0	28.5	2/10 (1)	− .7
400	1.0	33.0	4/15 (3)	− .3
600	1.0	29.9	5/19 (1)	−1.6
400	2.0	29.0	1/10	− .7
400	3.0	25.7	"	−1.7

* Mice surviving over 50 days were calculated as 50-day survivors in determination of avg survival time. Figures in parentheses indicate No. of complete regressions.

† Avg wt change from onset to termination of drug treatment.

ments on Rockland rat chow pellets and water *ad lib*. Drugs were injected intraperitoneally in isotonic saline; thioguanine was solubilized in dilute sodium hydroxide and neutralized to pH 7-8 with hydrochloric acid. Therapy was initiated 24 hours after tumor implantation and was continued once daily for 6 consecutive days (combination treatments given simultaneously). Tumor-bearing controls received injections of isotonic saline. Animals were weighed daily during treatments, and weight change from onset of therapy was used as an indication of drug toxicity. Survival time was used as the criterion of tumor inhibition. Mice surviving over 50 days and tumor-free animals were calculated as 50-day survivors in determination of average survival time. Complete regression of tumor growth was established by 2 criteria: drug-treated animals were maintained for 75 days to allow any surviving ascites cells to become evident; ascites tumor-free animals were then autopsied for presence of solid tumors in the peritoneal cavity. All gross nodules were examined histologically for presence of malignant cells.

Results. The effects of DL-glyceraldehyde and combinations of glyceraldehyde and thioguanine on survival time of mice bearing Sarcoma 180 are shown in Table I. Glyceraldehyde showed demonstrable effects at all dose levels tested; optimum dose was between 400-600 mg/kg of body weight. Addition of thioguanine to glyceraldehyde treatments appeared to increase slightly the effectiveness.

Table II shows the results obtained employing a combination of glyceraldehyde with azaserine and chloropurine. The effect produced by simultaneous administration of these agents was greater than that obtained at optimum doses of glyceraldehyde (Table I). Eleven out of 15 treated animals survived a minimum of 50 days; of these, 2 were complete regressions of tumor growth.

At a level of 400 mg/kg, glyceraldehyde produced a doubling of survival time of C3H mice bearing Hepatoma 134 cells, but it was without effect on survival of AKR mice bearing Mecca lymphosarcoma cells.

Discussion. The present results with glyceraldehyde are in direct contrast to previously reported(3) ineffectivenes of this compound against solid implants of the same tumor. Perhaps sufficient concentration of the inhibitor did not reach the subcutaneous implant or different strains of Sarcoma 180 were involved in the 2 experiments. The marked prolongation of survival time noted supported

TABLE II. Effects of DL-Glyceraldehyde in Combination with Azaserine and 6-Chloropurine on Survival Time of Sarcoma 180 Tumor-Bearing Mice.

Daily dose (mg/kg)			Avg survival (days)	No. 50-day survivors*	Avg wt (g)†
Glyceraldehyde	Azaserine	Chloropurine			
0	.0	0	10.9	0/14	+2.9
400	.0	0	25.6	0/10	+ .0
0	.2	40	30.2	3/15 (1)	+3.6
400	.2	40	44.5	11/15 (2)	− .9

* Mice surviving over 50 days were calculated as 50-day survivors in determination of avg survival time. Figures in parentheses indicate No. of complete regressions.

† Avg wt change from onset to termination of drug treatment.

the hypothesis that interference with glycolysis would inhibit neoplasia.

Combinations of glyceraldehyde, azaserine, and chloropurine were more effective than once daily treatments with glyceraldehyde or with azaserine and chloropurine. The results obtained were comparable to those observed using the same doses of azaserine and chloropurine given twice daily(4). The ability to demonstrate therapeutic synergy in the present system was limited by the potency of azaserine and chloropurine.

Summary. DL-glyceraldehyde prolonged survival time of mice bearing Sarcoma 180 or Hepatoma 134 ascites cells. Mecca lymphosarcoma ascites cells proved resistant to this agent. Combination of glyceraldehyde, azaserine, and chloropurine in the therapy of Sarcoma 180 produced a greater effect than that found using glyceraldehyde alone or with the dose level of azaserine and chloropurine employed.

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Site of Intracellular Antigen Production by Myxoviruses.* (25803)

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Application of fluorescent antibody technics to the study of multiplication of myxoviruses has suggested that the nucleoprotein component of influenza(1,2) and fowl plague(3, 4) viruses is produced within the nuclei of infected cells. The most complete study of this phenomenon is that by Breitenfeld and Schafer(3). From their experiments with chicken embryo fibroblasts infected with fowl plague virus they concluded that viral hemagglutinin is produced in the cytoplasm, whereas the nucleoprotein component of the virus particle is produced within the nucleus and later migrates to the periphery of the cell where it is combined with hemagglutinin to form complete, infectious virus particles. In our studies of the effect of Sendai virus on cells it was found that intranuclear antigen was not demonstrable by use of fluorescent antibody. Since this appeared to represent an important difference in characteristics of multiplication of viruses within the myxovirus group, the phenomenon was studied further. This paper compares the multiplication cycles of Sendai, mumps, and Newcastle disease viruses with that of influenza A virus in regard to intracellular site of antigen production.

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Materials and methods. Viruses. The PR-8 strain of influenza A, the Roakin strain of Newcastle disease virus (NDV), and the Enders strain of mumps virus were used. These were egg-adapted lines of virus cultivated by standard methods in the allantoic sac of chicken embryos. The Dunai strain of mumps virus(5) adapted to serial cultivation in human conjunctiva cells was used as virus from the 8th tissue culture passage. The Sendai strain of para-influenza I virus was cultivated in the allantoic sac of chicken embryos(5). A line of Sendai virus adapted to serial cultivation in human conjunctiva cells was used as virus from the 7th tissue culture passage. *Cell cultures.* Primary cultures of chick embryo lung cells were prepared from lungs of 11-day-old embryos(6). They were grown in Scherer's medium with addition of 5% chick embryo extract and 15% horse serum and were maintained in this medium after inoculation. Human conjunctiva cells (Chang) were grown in serial culture in Eagle's basal medium containing 20% horse serum and after inoculation