planted tumors surgically and harvesting lymphocytes 7 to 14 days afterwards, or (iii) by inoculating the mice once with tumor cells that had been given 12,000 to 24,000 roentgens of x-irradiation and harvesting the lymphocytes from 10 to 80 days afterward. The irradiated cells were derived from the primary tumors or from their first or second transplant generations. Finally, in eight experiments, primary MCA-induced tumors were surgically removed, their cells were explanted, and the lymphocytes were tested on the autologous target cells 3 to 8 days afterward.

Table 1 shows that there was a lower colony number in groups receiving lymphocytes from donors immunized to the target tumor than in groups given lymphocytes from either untreated donors or from donors immunized to another syngeneic MCA sarcoma. The results with donors immunized to the syngeneic tumors agree with the demonstration in vivo that individual MCA tumors have specific antigens which do not cross-react (*2*). They also agree with a report by Rosenau and Morton (*6*) who found approximately 30 percent fewer living cells in cultures of transplanted MCA tumors which had been treated with specifically immune lymphocytes than in controls treated with lymphocytes immunized against the antigens of another MCA tumor. However, the CI assay appears to be more sensitive, since differences of 40 to 90 percent were generally observed in our study.

Autologous lymphocytes were strongly inhibitory when derived from operated mice that had carried MCA tumors (Table 1), as was seen in all of eight experiments performed. The demonstration of immunity to the tumors in the autologous host shows that either the tumor exists in vivo in spite of a capacity of the lymphoid cells to kill it in vitro or such a capacity is gained during the 3- to 8-day interval between tumor removal and harvesting of lymphocytes. This may be used as a starting point to analyze host immune mechanisms against autologous tumor cells.

Two of three tumors induced by Dictabelt plastic film were similar to the MCA sarcomas in being sensitive to the CI effect of specifically immunized lymphocytes. The immunization had been performed by inoculating the lymphocyte donors with either heavily x-irradiated or untreated tumor cells; in the latter case, the lymphocytes had been harvested before tumor appearance. The CI effect with these film-induced tumors was, however, smaller than that obtained with MCA sarcomas. This agrees with the lower antigenicity of the film-induced tumors in vivo (*1*).

The addition of phytohemagglutinin was not necessary to demonstrate colony inhibition with immune lymphocytes sensitized against target-cell histocompatibility antigens. On the other hand, PHA is needed to demonstrate cytotoxicity with untreated lymphocytes (*7*).

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Food and Water Intake after Intrahypothalamic Injections of Carbachol in the Rabbit

Abstract. *Direct chemical stimulation with carbachol in the hypothalamus of the rabbit caused a significant increase in food intake, as a function of place stimulated and concentration. Lower doses of carbachol injected near the supraoptic nucleus produced an increase in drinking without an increase in eating.*

Direct injection of carbamylcholine chloride (carbachol) in certain areas of the brains of rats increases intake of water. Application of levarterenol bitartrate (*l*-norepinephrine) to identical sites induces increased food intake. These results have led to the hypothesis that feeding is mediated by an adrenergic mechanism, and that drinking is mediated by a cholinergic mechanism (*1*).

We tested the generality of effects of direct stimulation by these substances in the New Zealand albino rabbit. Chemicals dissolved in mammalian Ringer-Locke solution were used to control doses and *p*H, and to reduce possible damage at the injection site. Crystalline carbachol has the same effect on rats as solutions have (*2*).

Rabbits were implanted with cannulas that were made from tubing (22-gauge) cut to 24-mm lengths and held by a piece of Amphenol connector strip (*3*). A Trent-Wells stereotaxic instrument adapted for rabbits was used, and the cannulas were localized by use of an atlas for the rabbit brain (*4*). Placements were verified histologically. Injections were made by inserting an inner cannula (28-gauge tubing) into the outer cannula. Test solutions were delivered with a microsyringe attached to the inner cannula with polyethylene tubing. The rabbits had free access to water and dry food pellets. Beginning 1 week after the operation, we tested all animals every other day.

Various doses of carbachol were administered to each animal at least twice in different sequences (Fig. 1). Eating began 30 seconds to 3 minutes after

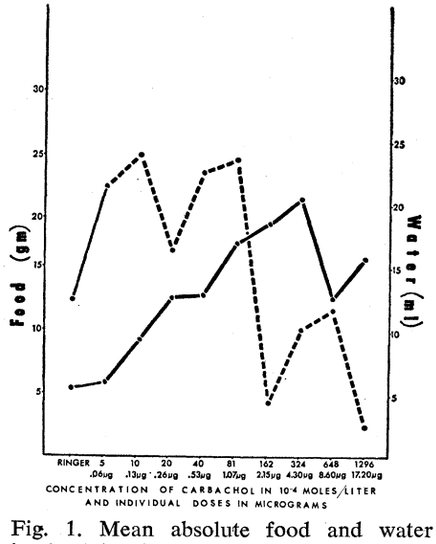

Fig. 1. Mean absolute food and water intake following injections of various concentrations of carbachol ($5 \times 10^{-4}M$ to $1296 \times 10^{-4}M$) and Ringer's solution. These data were obtained from eight animals. The solid line indicates food intake, and the dashed line indicates water intake.

injection of drugs in sufficient concentration to cause increased intake. At lower concentrations, and only with injections near the supraoptic nucleus, drinking began between 15 and 45 minutes after injection. At the highest concentrations, administered in all areas which produced eating, animals frequently made low grunting sounds and pounded their hind legs on the floor of the cage. There is a significant increase in food intake as the amount of carbachol injected is increased, as shown by the linear component of the trend of means ($P < .01$ $F = 32.09$, $df = 1,56$); there is significantly higher food intake at the optimum dosage than at the lower or higher dosages, as shown by the quadratic component of the trend of means ($P < .01$, $F = 22.85$, $df = 1,56$). Water intake at the lower doses is significantly greater than at the higher doses, as shown by the linear component of the trend of means ($P < .01$, $F = 19.95$, $df = 1,56$). The higher-order comparisons for water intake are not significant.

Our data were obtained from animals stimulated in the lateral hypothalamus, lateral preoptic area, and the area dorsal and anterior to the medial preoptic area. These areas were not differentiable by amount eaten. Animals with increased drinking at the low doses had the stimulating cannula relatively low between the medial and lateral preoptic areas near the supraoptic nucleus. Placements where stimulation yielded no significant changes in eating or drinking were in the lateral part of the anterior hypothalamus, fornical area, and the medial preoptic area. The close proximity of cannulas yielding effects and of those yielding no effects indicates a small amount of effective spread of the injected solutions. Animals that ate more with carbachol were tested with $1536 \times 10^{-4} M$ *l*-norepinephrine, but none ate or drank significantly more. Some animals were tested with injections of hypertonic saline, but no effects were observed.

In the rabbit, the optimum dose that effects eating is 4.3 μg. The optimum dose that causes drinking in the rat is 0.43 μg (*2*). These doses are approximately 1.0 μg per kilogram of body weight for both animals. The dose-response curve for eating after injections of carbachol in the rabbit shows an inverted U function (also shown for drinking in the rat), which indicates that there is an optimum dose; concentrations above and below this optimum are less effective. Rabbits and rats exhibit other behaviors at the highest concentrations of drugs which interfere with eating and drinking.

An increase in eating would normally be accompanied by an increase in drinking. At dosages producing the greatest amount of eating, there is also a decrease in drinking (Fig. 1). This effect was not limited to stimulation in any specific area. In view of the fact that these scores are for a period of 1 hour, this result is probably not due to a lack of time available to observe increased drinking. The rabbits were usually resting or grooming for the last 15 to 30 minutes of the observation period. This decrease in drinking may be similar to the reciprocal inhibition reported by Grossman (*1*), who observed that carbachol in rats inhibits eating and that *l*-norepinephrine inhibits its drinking.

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Protein Metabolism in the Developing Brain: Influence of Birth and Gestational Age

Abstract. *Incorporation of carbon-14–labeled phenylalanine into brain protein of newborn pigs falls sharply within 24 hours after birth. This decrease is related to the time of birth rather than the gestational age of the piglets, although the latter is also associated with a gradual decrease in brain protein synthesis.*

A number of studies have indicated that brain protein synthesis, as determined by incorporation of isotopically labeled amino acids into protein fractions, decreases with age in young animals (*1*). The major decrease in protein synthesis seems to occur in the first days of life (*2*). However, the information available has been unclear as to whether this decrease is due to maturational factors determined by the gestational age of the animal, or to changes associated with birth itself. This report describes experiments suggesting that factors associated with birth result in a sharp decrease in amino acid incorporation into brain protein.

Miniature pigs were utilized in all experiments (*3*). The gestational period of the pig is 114 days, with relatively little variation. The piglets were procured by hysterectomy (*4*) at different gestational ages or allowed to farrow at term. The newborn piglets were immediately placed in a Gordon-Armstrong incubator maintained at 33°C throughout the experimental period. They were allowed to feed ad libitum on an artificial sow's milk replacement formula (SPF-lac, Borden) with varying degrees of success. Several farrowed litters nursed their dam in an ordinary stall.

Tracer doses of uniformly labeled L-phenylalanine-^{14}C (specific activity 300 mc/mmole) were administered intraperitoneally to the piglets at a uniform dose of 20 mc/kg. One hour after injection, the animals were exsanguinated and the brain removed within 2 minutes. The cortical mantle was removed for preparation of trichloroacetic acid soluble (free amino acid) and insoluble (protein) fractions (*5*). Radioactivity in the fractions was determined by conventional liquid scintillation techniques (*6*).

Phenylalanine incorporation into brain protein falls off sharply after birth (Fig. 1). This occurs irrespective of gestational age, as demonstrated by the even more abrupt fall occurring when the newborn animals were delivered by hysterectomy at varying gestational ages. The decreased rate of incorporation persisted even though the naturally delivered piglets quickly established nursing habits and established weight gains by 24 to 48 hours of age. The more immature (100 to 103 days) of the hysterectomy-delivered piglets were feeble and often fed little during the postnatal period. However, many of the piglets delivered at 110 days of gestational age fed vigorously and sustained little or no weight loss during the first