thesis. The big GH component may (*16, 17*) or may not (*18*) show decreased binding activity in the radioreceptor assays. Discrepancies between estimates of immunoreactive hormone and expected biologic activity may result from a disproportionate increase in the big GH component, although other explanations, such as a monomeric GH with reduced or absent biological activity (*17*), must be considered. Such an increase could be produced by hypothalamic or other extrapituitary agents which may differentially alter the release of newly synthesized big and stored small GH (*15, 19*). Finally, a subcellular defect along the path from hormone synthesis to storage could result in an enhanced secretion of big GH, since release of this form appears to be related to concurrent GH synthesis.

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Endogenous Cyclic Adenosine Monophosphate in Tissues of Rabbits Fed an Atherogenic Diet

Abstract. *Rabbits fed a high cholesterol diet experienced a significant increase in plasma adenosine 3′,5′-monophosphate (cyclic AMP), which was simultaneous with the increase in plasma cholesterol. The content of cyclic AMP in atherosclerotic lesion areas of rabbit aortic intima-media was significantly higher (0.24 picomole per microgram of DNA) than that in adjacent nonlesion areas or in aortic intima-media from control animals (0.09 picomole per microgram of DNA). The cyclic AMP content of heart, liver, skeletal muscle, and diaphragm showed no significant elevation in animals fed cholesterol.*

Adenosine 3′,5′-monophosphate (cyclic AMP) has been suggested as a regulator of lipid metabolism, membrane transport, and cell proliferation. Several investigators have reported its action on these cell processes to be either stimulatory or inhibitory, depending on the concentration used (*1*).

The selective susceptibility of focal regions of the arterial vasculature to the development of atherosclerotic lesions is well established. This selective susceptibility, coupled with the fact that atherosclerotic lesions are characterized by an increase in cell proliferation and lipid accumulation, suggested that the cyclic AMP in the plasma of animals on atherogenic diets might vary from that of controls. A study of rabbits fed cholesterol showed such an increase in the cyclic AMP concentration of plasma and in lesion areas of the aortic intima-media.

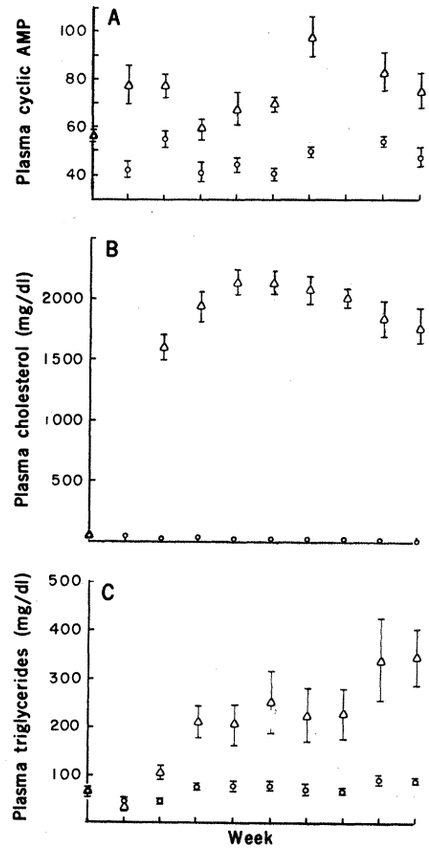

Fig. 1. Cyclic AMP, cholesterol, and triglyceride in plasma of rabbits on high cholesterol (△) and control (○) diets from one experiment. Data are given as the mean ± the standard error of the mean.

For each of two experiments, 16 male New Zealand white rabbits, 6 to 8 weeks of age, were randomly divided into two groups of eight animals each. Atherosclerotic lesions were induced in one group by feeding them Purina Rabbit Chow supplemented with 2 percent cholesterol in a corn oil vehicle. The control group was maintained on Purina Rabbit Chow only, and water was freely available to both groups. Plasma concentrations of cyclic AMP (*2*), cholesterol (*3*), and triglycerides (*4*) were determined weekly on both groups of rabbits. After 9 weeks, all animals were killed by cervical fracture. Portions of heart, liver, skeletal muscle, and diaphragm were quickly excised from randomly selected animals, briefly washed in saline, teased into small pieces, and fixed in 6 percent trichloroacetic acid for 10 minutes. The entire aorta was removed from each animal. Approximately 60 to 70 percent of the total aortic surface of rabbits on the cholesterol-supplemented diet was covered with macroscopically identifiable lesions which were stainable with Sudan dye. This extent of involvement precluded meaningful correlation between plasma cyclic AMP levels and the severity and extent of lesions. In addition, attempts at correlating the plasma concentrations of cyclic AMP and cholesterol in individual rabbits, monitored over the 9-week period, with the severity of lesions at the time the animals were killed proved inconclusive. For consistency, only the upper half of the thoracic portion of the aorta was fixed in trichloroacetic acid. After fixation, the adventitia was stripped, and macroscopically identified lesion areas were carefully dissected from

Table 1. Endogenous tissue levels of cyclic AMP. Values are expressed as the mean ± standard error of the mean of quadruplicate determinations. The number of animals is indicated in parentheses.

Tissue	High cholesterol diet (pmole/μg of DNA)	Control diet (pmole/μg of DNA)
Aorta		
Lesion	0.24 ± 0.03 (6)*	
No lesion	0.09 ± 0.009 (6)	0.09 ± 0.004 (8)
Heart	0.46 ± 0.06 (7)	0.40 ± 0.02 (6)
Liver	0.30 ± 0.03 (12)	0.32 ± 0.04 (10)
Skeletal muscle	0.63 ± 0.07 (11)	0.67 ± 0.10 (7)
Diaphragm	0.99 ± 0.12 (5)	0.85 ± 0.11 (5)

* $P < .001$.

areas without lesions. Those aortas having an insufficient amount of lesion in the designated area were withdrawn from analysis. All tissues were lyophilized and stored at −20°C until used for determination of endogenous cyclic AMP content as described by Steiner and co-workers (*5*). Results obtained from tissues fixed in liquid nitrogen were identical to those obtained with trichloroacetic acid fixation. Cyclic AMP is reported as picomoles per microgram of tissue DNA (*6*).

Plasma cyclic AMP, cholesterol, and triglyceride concentrations from one experiment (eight experimental, eight control animals) are shown in Fig. 1. Cyclic AMP concentrations were not determined for week 7 because of a technical error. There was a significant rise in cyclic AMP in the plasma after week 1 of the cholesterol diet, coincident with the expected increase in plasma cholesterol. The plasma cyclic AMP concentration of cholesterol-fed animals remained significantly elevated ($P < .001$), with some week-to-week variation, throughout the course of the experiment. However, it did not equal the 43-fold overall increase in plasma cholesterol or the 4-fold overall increase in plasma triglycerides. Data from the other experiment supported these conclusions. Such a time-independent but significant increase in the concentration of cyclic AMP is consistent with its role as a "second messenger," that is, a transducer of physiological signals designed to elicit specific biochemical responses without itself effecting the biochemical response (*7*).

The change in the circulating cyclic AMP level demonstrated in the plasma suggested the possibility of a change in the tissue concentration of cyclic AMP. Measurement of endogenous cyclic AMP in several tissues from randomly selected animals from both experiments (Table 1) showed a significantly higher concentration in atherosclerotic lesion areas in aortic intima-media than in adjacent areas where there were no lesions ($P < .001$). Likewise, the cyclic AMP in lesion areas was higher than that in the aortic intima-media of control rabbits. There was no significant elevation of cyclic AMP in heart, liver, skeletal muscle, or diaphragm of the animals on a high cholesterol diet. The specificity of the increase for aortic lesion areas is of special interest in light of the fact that, at 9 weeks, the liver and aorta of animals on the high cholesterol diet had large accumulations of lipid.

The increase in cyclic AMP concentration in plasma and lesion areas is compatible with a disease process characterized by increased lipid permeability and increased cell proliferation. Strange and co-workers (*8*) noted an elevation of plasma cyclic AMP concentration during the first few hours after the onset of symptoms of acute myocardial infarction in man. Bricker and Levey (*9*), using a mammalian liver system, have presented evidence that cyclic AMP may be involved in regulating acetyl–coenzyme A incorporation during de novo fatty acid and cholesterol synthesis. The significance of cyclic AMP concentrations in atherosclerosis has not yet been established. It may be involved in the pathophysiology of the disease, or it may be a secondary consequence. The observation is important because of the regulatory function of cyclic AMP in many cellular processes and because, in plasma, the concentration increase can be demonstrated as early as the increase in plasma cholesterol.

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Measles Virus: An Unwanted Variant Causing Hydrocephalus

Abstract. *Mutagenization of measles virus with proflavine produced a temperature-sensitive mutant capable of inducing hydrocephalus following intracranial inoculation of newborn hamsters. Hydrocephalus was not produced by the parental strain or by other measles virus mutants. Thus, mutants can be the causative agents of disease not associated with the parental strain. The results dictate caution in the use and distribution of experimentally induced virus variants.*

Intracranial inoculation of suckling hamsters with mumps virus not adapted to replication in the brain resulted in the induction of hydrocephalus (*1*). Hydrocephalus, characterized by aqueductal stenosis, was similarly produced with high frequency by intracranial inoculation of influenza virus Ao and parainfluenza virus type 2; but it was not produced by measles virus (*2*). We now report that a mutant strain of measles virus induced hydrocephalus whereas the parental strain did not.

Temperature-sensitive (ts) mutants of measles virus were isolated after the virus had undergone chemical mutagenesis (*3*) and were examined for their neurovirulent potential in the newborn golden Syrian hamster (*4*). During these initial experiments, it was observed that, of eight hamsters autopsied between 18 and 49 days after intracranial inoculation with ts mutant G (tsG), six showed hydrocephalus char-