CENTRAL CONTROL OF FOOD INTAKE IN THE DOMESTIC FOWL

by

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(ABSTRACT)

This study was initiated to determine if factors exist in the blood or cerebrospinal fluid (CSF) of the domestic fowl that act upon the central nervous system to control food intake.

Plasma collected from free-feeding and 24-hour fasted leghorn cockerels was lyophilized, reconstituted to 2, 4, or 5 times the original concentration, and injected, via a stereotaxically implanted 23 gauge stainless steel guide cannula, into the lateral ventricle of free-feeding leghorn cockerels. Food intake was significantly reduced following injection of 2, 4, and 5 times normal concentration of plasma from free-feeding birds. Plasma from fasted birds did not alter food intake regardless of concentration, but did significantly reduce water intake when concentrated to five times normal.

A similar study was conducted with fractions of plasma of different molecular weight ranges. Plasma collected from free-feeding cockerels was partitioned by gel filtration into the following molecular weight fractions: >5000 molecular weight, <5000 molecular weight, 1500-5000 molecular weight, and <1500 molecular weight. The fractions

were lyophilized and reconstituted to four times the original concentration and injected into the lateral ventricle of free-feeding leghorn cockerels. Food intake was significantly decreased by the <5000 and <1500 molecular weight fractions, whereas water intake was not affected. The 1500-5000 molecular weight fraction and the fraction above 5000 did not affect food or water intake.

To determine if this food intake inhibiting factor existed in the cerebrospinal fluid (CSF) of the domestic fowl, CSF was collected from free-feeding and 24-hour fasted broilers and injected into the lateral ventricle of leghorn and broiler cockerels. Food intake was not affected by either the normal or four-times normal concentration of CSF collected from free-feeding or 24-hour fasted broilers. Water intake was significantly increased in the leghorn and broiler birds receiving the four times normal concentration of CSF collected from 24-hour fasted birds, but was not affected in the birds receiving CSF collected from the free-feeding donors.

It appears, therefore, that a food intake inhibiting factor exists in the plasma of the free-feeding domestic fowl that does not exist in the CSF.

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INTRODUCTION

The intake of food provides living organisms with the basic nutrients required for maintaining life. Regulation of the amount and quality of food consumed plays an integral part in an animal's homeostatic mechanisms. Overconsumption can lead to obesity while underconsumption can result in nutritional deficiencies. Elucidation of the mechanisms that regulate food intake would be of value medically for the treatment of diseases ranging from obesity to anorexia nervosa and financially for the enhancement of production in domestic animals.

There are numerous theories regarding the control of food intake, most of which are based on the concept of a "satiety center" in the ventromedial hypothalamus (VMH), and a "feeding center" in the lateral hypothalamus (LH) (Powley et al., 1980). Food intake has been reduced by stimulating the VMH of the rat (Kranse,1962) or lesioning the LH of the rat (Anand and Brobeck, 1951), domestic chicken (Smith, 1969), and White Throated Sparrow (Kuenzel, 1972). A stimulation of food intake occurred following lesioning of the VMH of the rat (Brobeck et al., 1943), domestic chicken

(Lepkovsky and Yashuda, 1966; Burkhart et al., 1983) and White Throated Sparrow (Kuenzel and Helms, 1970), or following anesthetization of the VMH (Epstein, 1960) and stimulation of the LH (Miller, 1960) of the rat.

Although many theories have been proposed, the mechanisms by which the hypothalamus may regulate food intake are not well defined. Five of these theories--the glucostatic (Mayer, 1955), lipostatic (Kennedy, 1953), aminostatic (Mellinkoff et al., 1956), ionostatic (Myers et al., 1972), and the osmostatic (McLeary, 1953) are based on a feedback mechanism through the monitoring of a given nutrient in the body. Alterations in plasma nutrient levels may be monitored centrally (Kennedy, 1953; Mayer, 1955) or peripherally as suggested by the dual chemical profile theory for the regulation of food intake (Myers, 1975). The peripheral theory involves the monitoring of chemical substances that are altered by nutritional balance, i.e., carbohydrates, lipids, amino acids, and hormones. The central profile, on the other hand, is represented by the neurochemical events occurring within the brain that are involved in initiating and terminating feeding. This suggests that the activity of the central profile is the result of changes in the peripheral profile.

It has been suggested that a decrease in the level of pyruvate in the liver causes hepatocyte receptors to increase their firing rate and send signals to the brain which initiates eating (Russek, 1981). The GI tract has also been considered in the integration of food intake regulation. Smith and Gibbs (1979) and Deutsch (1978) suggested that neural and/or humoral signals arise from the stomach and intestine that inhibit food intake. Other mechanisms may be involved because McHugh (1979) indicated that caloric intake may be controlled by distension of the stomach, and Polin and Wolford (1973) hypothesized that regulation of food intake in the chicken was related to the rate of fill, the capacity, and the rate of discharge of food from the crop.

Although the actual link between the peripheral and central regulatory systems has not been established, it is becoming more apparent that a blood-borne or CSF-borne substance may be responsible for the initiation or suppression of feeding. Research on the involvement of a "humoral" factor in the regulation of food intake has been reported for a number of species. Hervey (1959) lesioned the VMH of one member of a pair of parabiotic rats. The result was a twofold increase in body weight of the lesioned rat and simultaneous loss of weight in the non-lesioned

partner. Post-mortem findings following 2-3 months of parabiosis showed an almost complete loss of body fat in the non-lesioned rat while the VMH lesioned member had 4-5 times as much body fat as a single member of a non-lesioned pair. This was the first report suggesting the existence of a humoral satiety factor which could be passed from one animal to another. Later studies of a similar design have provided confirming results (Hervey, 1959; Han et al., 1963; Fleming, 1969; Kasermann and Stamm, 1975). In 1977, Parameswaran et al. induced feeding in one member of parabiotic rats by electrically stimulating the LH. As before, the animal stimulated to eat became obese while the non-stimulated partner became thin. Measurements of blood glucose, insulin and glucagon of both partners suggested that these blood constituents were not responsible for satiety.

In order to eliminate some of the problems with the parabiotic experiments, i.e., the obese partner restricting the feeding of the lean partner, researchers investigated the possibility of transfusing blood between satiated and fasted animals. Davis et al. (1969) mixed the blood of a satiated (ad lib feed) and 24-hour fasted rat and readministered the mixed blood into the rats. The result was a 50 percent reduction in food intake in the fasted rat. Davis et al. (1971) measured the volume of food ingested by

22-hour food-deprived rats immediately following blood transfusions from donor rats deprived of food for 0, 1, 2, 3, 4, or 5 hours. The amount of food consumed by the recipient following transfusion was inversely proportional to the length of deprivation of the donor rat. Blood from donors deprived of food for 4 hours caused only a slight reduction in the food intake of the recipient rats, suggesting that the satiety factor may have dissipated. The 4 hour period coincides well with the 4 hour intermeal interval reported for ad lib fed rats (Balagura and Coscina, 1968). The transfusion of blood from rats deprived for 5 hours resulted in food intake 127 percent of controls. This suggested the presence of a "hunger hormone" which stimulated feeding in rats (Davis et al., 1971).

Work with sheep has also provided evidence for the possibility of a hunger hormone which stimulates feeding. Seoane et al. (1972) cross-circulated blood between satiated and 5-hour fasted sheep. Following cross-circulation, the satiated sheep ate 48 percent more feed than the control group, and hungry sheep consistently consumed less than the control group. Seoane and Warner (1971) had previously reported that hungry sheep receiving blood from satiated sheep exhibited a 40 percent reduction in food intake although the intake of the satiated sheep

receiving blood from hungry sheep was not significantly affected.

In addition to blood-borne factors, CSF-borne factors involved in food intake regulation have been investigated. The structures of the hypothalamus form the floor and a portion of the wall of the third ventricle. This intimate relationship provides considerable opportunity for substances carried in the cerebrospinal fluid (CSF) to pass into specific hypothalamic regions. Conversely, substances released from the hypothalamic nuclei may pass into the CSF. A technique developed by Myers (1967) allowed CSF to be transferred between the brains of conscious monkeys. Initial experiments by Myers (1969) showed no effect on food intake in monkeys following lateral ventricular infusion with .5 ml of CSF from a food deprived monkey, although the satiated recipient did increase feeding. Injection of CSF from a hungry monkey directly into the LH of a satiated monkey increased food intake. Using push-pull cannulas directed towards a variety of areas within the hypothalamus it was found that a factor which inhibited food intake was released predominantly in the region of the VMH whereas an active feeding factor was released in a less discrete area within the hypothalamus (Yaksh and Myers, 1972).

Martin et al. (1973) reported a five-fold increase in food intake in sheep during the first 15 minutes following a lateral ventricular injection of 1 ml of CSF from a fasted donor. Similar results were obtained by injecting .5 ml of CSF from fasted sheep into the third ventricle. Injection of CSF from satiated donors into the lateral ventricle of fasted sheep resulted in a 20 percent decrease in food intake.

Little work has been done to characterize those factors affecting food intake. Parameswaran et al. (1977) measured the insulin, glucagon and glucose levels in parabiotic rats following electrical stimulation of the LH of one partner and concluded that those blood constituents were not responsible for the reduced feeding of the lean partner. Myers (1975) used gas chromatography-mass spectrometery analysis to determine differences in the CSF between satiated and fasted cats. Analysis of the CSF revealed 51 substances were altered by the state of feeding. Many of the substances are quite ubiquitous and nutrient related.

In summary, considerable documentation exists for the presence of a factor in the blood and CSF that is altered by nutritional status and can be passed to another animal where it will simulate the nutritional status of the donor. Transfusion of blood between fasted and satiated rats caused

an increase in feeding in the satiated rats and a decrease in feeding in fasted rats (Davis et al., 1969). Similar effects were seen following cross-injection of CSF between satiated and fasted sheep (Martin et al., 1973) and monkeys (Myers, 1969).

The information regarding the link between the GI tract and the food intake regulatory centers in the brain is somewhat limited for mammals and very limited for Aves. Thus, the objectives of this project were;

1) To determine if neurohumoral factors regulating food intake exist in the blood or in the CSF of the domestic fowl.

2) To characterize such neurohumoral factors.

Chapter 2

ALTERATION OF FOOD INTAKE FOLLOWING INTRACEREBROVENTRICULAR ADMINISTRATION OF PLASMA FROM FREE-FEEDING DOMESTIC FOWL

The presence of a blood-borne factor that can alter food intake has been well-documented. Cross-transfusion of blood between fasted and sated rats resulted in a 50 percent reduction of food intake in the fasted rats (Davis et al., 1969). Transfusion of blood from rats deprived of food for 5 hours stimulated food intake to 127 percent of controls (Davis et al., 1971). Cross-circulation of blood between fasted and sated sheep reduced food intake by 40 percent in the sheep receiving blood from sated donors (Seoane et al., 1971) and increased food intake 48 percent in the sheep receiving blood from a fasted donor (Seoane et al., 1972).

A variety of techniques has been utilized to demonstrate the involvement of the ventromedial hypothalamus (VMH) and lateral hypothalamus (LH) in the regulation of food intake. Food intake was stimulated in rats by lesioning (Brobeck et al., 1943) and anesthetizing (Epstein,1960) the VMH or stimulating the LH (Miller,1960). On the other hand, food intake was reduced by stimulating

the VMH (Kranse,1962) or lesioning the LH (Anand and Brobeck,1951). Lesioning the VMH of the chicken (Lepkovsky and Yashuda, 1966) and White Throated Sparrow (Kuenzel and Helms, 1970) produced hyperphagia and obesity, whereas lesioning the LH in the chicken (Smith, 1969) and sparrow (Kuenzel, 1972) resulted in hypophagia and weight loss. Intracerebroventricular (ICV) injection of a narrow molecular weight range fraction of human serum into 96-hour fasted rats has also been shown to reduce food intake (Knoll, 1979). It appears, therefore, that the hypothalamus controls food intake by monitoring the levels of various plasma components.

The objective of this study was to determine if a blood-borne factor exists in the domestic fowl which can alter food intake when injected into the lateral cerebral ventricle. The effect of ICV injection of plasma on water intake was also determined.

MATERIALS AND METHODS

Animal preparation. Leghorn cockerels were raised in electrically heated battery brooders with food and water available ad libitum. At 8 weeks of age the birds were transferred to individual cages. Following sodium pentobarbital anesthesia (25 mg/kg), a 23 gauge stainless steel guide cannula was stereotaxically implanted into the

right lateral ventricle by the procedure of Denbow et al., (1981) . The guide cannula was occluded with a 27 gauge stylet between injections. Following a 3 day recovery period, validation of cannula location was determined by monitoring the colonic temperature response to an injection of 67 µg of norepinephrine in 10 µl of artificial cerebrospinal fluid[1] (aCSF) administered via the guide cannula. Only those birds exhibiting a decrease in body temperature of 1.0°C or greater were used in the experiments.

Plasma Preparation. Blood was collected via cardiac puncture from free-feeding or 24-hour fasted cockerels. The blood was centrifuged at 3000 g for 20 minutes and the plasma was collected and stored at -20°C. Plasma to be concentrated was lyophilized and reconstituted with distilled water to 2, 4, or 5 times (2X, 4X, or 5X) the original concentration and stored at -20°C (appendix B).

Injection Procedure. Injections were made with a 27 gauge stainless steel injection cannula connected to a 10-µl Hamilton syringe via PE-50 tubing. In each experiment the birds received either 10 µl of aCSF, plasma collected from free-feeding birds (Fed-plasma), or plasma collected

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[1]The aCSF consisted of 155 mM Na^+, 2.5 mM Ca^{++}, 3.7 mM K^+, 2.1 mM Mg^{++}, 140 mM Cl^- and 23 mM HCO_3^- (Anderson and Hazlewood, 1969).

from 24-hour fasted birds (Fasted-plasma). The plasma was administered at 1, 2, 4, or 5 times normal concentration (see Table 1). Food and water intakes were monitored at 15-minute intervals for the first hour, 30-minute intervals for the second hour, and hourly thereafter.

Statistical design and analysis. A repeated Latin Square design (Snedecor and Cochran, 1980) was utilized. In Experiments 1, 2, and 3 non-orthogonal contrasts were used to make comparisons of cumulative food and water intake between the control and treated birds at each time period. Linear contrasts were used to compare food and water intake between birds receiving plasma from free-feeding or 24-hour fasted birds in Experiment 4. Bonferroni F values ($p \leq .05$) were used in determining significance(Games, 1972).

RESULTS

Experiment 1. Injection of 10 µl of Fed-plasma or Fasted-plasma of normal concentration (1X) into the lateral cerebral ventricle did not significantly alter food or water intake from that of the controls (Table 2). Although not significant, birds receiving plasma from fed donors tended to consume less food than did the control birds. This observation was the basis for the subsequent experiments in which the plasma was concentrated prior to injection.

Experiment 2. Food intake of the birds receiving the 2X concentration of Fed-plasma was significantly less than that of the control birds at 30, 45, 60, and 90 minutes post-injection. The 2X Fasted-plasma, however, did not significantly alter food consumption. Water intake was not significantly altered by either treatment (Table 3).

Experiment 3. Injection of the 5X concentration of Fed-plasma significantly reduced food intake at 45, 60, 90, and 120 minutes following injection without any significant alteration in water intake. Those birds receiving 5X Fasted-plasma consumed significantly less water than did the controls at 45 and 180 minutes post-injection, although food intake was not affected (Table 4).

Experiment 4. Birds receiving Fed-plasma exhibited a dose dependent reduction in food intake from 15 to 240 minutes following injection (Table 5); the Fasted-plasma did not significantly alter food intake. Water intake was not affected by any treatment.

DISCUSSION

The ICV injection of the concentrated plasma collected from free-feeding birds caused a significant decrease in food intake. This response is in agreement with earlier studies where it was reported that a factor exists in the blood of the rat (Davis et al., 1969) and sheep (Seoane and

Warner, 1971) that reduces food intake when transfused into a fasted animal. In our experiment, plasma collected from fasted birds did not stimulate food intake; however, in the rat (Davis et al., 1971) and sheep (Seoane et al., 1972) a stimulation of food intake was reported following transfusion with blood collected from a fasted donor.

The reduction in water intake resulting from the 5X concentration of Fasted-plasma is probably not due to the hyperosmolarity of the concentrated plasma, since the normal response to hypertonic conditions within the brain would be a stimulation of water intake (Verney, 1947). Osmolarities of the 4X concentration of Fed-plasma and Fasted-plasma were 1401 mOS and 1460 mOS, respectively. This difference was probably not large enough to account for the disparate food intake response of birds receiving Fed-plasma and Fasted-plasma. Since water intake was not reduced by either the 2X or 4X Fed-plasma, it can be assumed that the effect of the treatments was specific for food intake.

The nature of the factor(s) present in the plasma that reduced food intake when injected ICV is unknown. Numerous endogenous substances have been shown to decrease food intake when injected ICV. A hypophagic condition was reported in the rat (Avery and Callisher, 1982; Stuckey and Gibbs, 1982) and pig (Parrot and Baldwin, 1982) following

ICV administration of bombesin. Similarly, ICV injection of cholecystokinin reduced food intake in the rat (Maddison, 1977) and chicken (Denbow and Myers, 1982). Nutrient-related blood components have also been shown to be involved in regulating food intake. Infusion of 6% glucose into the lateral ventricle of domestic fowl reduced food intake for up to 3 hours post-injection (Matei-Vladescu et al., 1977).

The lack of a food intake response following ICV injection of Fasted-plasma may have been caused by the reduction or complete elimination of some factor(s) in the plasma due to nutritional status. The concentration of the factor in the plasma may decrease in proportion to the length of time the animal has gone without feeding. This type of response has been demonstrated in the rat (Davis et al., 1971). The amount of food consumed by a sated recipient rat following transfusion with blood from a fasted donor rat was inversely proportional to the length of food deprivation of the donor rat.

The results of our study suggest that a satiety factor, which can be detected by the brain, exists in the plasma of the free-feeding domestic fowl. Whether this factor originates from the gastrointestinal tract as a secretion, e.g., cholecystokinin or bombesin, or from the absorption of nutrients, e.g., glucose, remains to be elucidated. The

plasma concentration of this factor appears to be related to the nutritional status of the bird or the length of time the bird has been fasted.

Table 1. Experimental outline

Treatments by Experiment			
Experiment			
1	2	3	4
Control[1]	Control	Control	Control
1X Fed-plasma	2X Fed-plasma	5X Fed-plasma	2X Fed-plasma
1X Fasted-plasma	2X Fasted-plasma	5X Fasted-plasma	4X Fed-plasma
			2X Fasted-plasma
			4X Fasted-plasma

[1]Artificial cerebrospinal fluid.

Table 2. Mean cumulative food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of normal (1X) concentration plasma from free-feeding or 24-hour fasted birds

Treatment*	Time post-injection (min) 15	30	45	60	90	120	180	240
	----------Food intake (g)----------							
Control[1]	1.2	2.2	3.7	4.7	6.2	8.3	12.3	15.6
Fed-plasma 1X	.6	1.6	2.8	3.5	5.4	7.2	11.0	14.9
Fasted-plasma 1X	1.7	2.5	4.1	4.7	6.9	9.1	12.0	16.2
SEM[2]	.3	.4	.5	.6	.7	.8	.8	1.0
	----------Water intake (ml)----------							
Control[1]	1.1	2.2	3.3	4.4	6.1	8.6	12.8	18.9
Fed-plasma 1X	.3	1.1	2.5	3.3	5.3	9.7	15.0	21.1
Fasted-plasma 1X	.6	1.7	2.8	3.9	5.3	8.9	13.3	20.1
SEM[2]	.5	.7	.9	1.0	1.1	1.5	2.3	3.1

[1]Artificial cerebrospinal fluid.
[2]Standard error of the mean (n=18).
*No treatment was significantly different from control ($P \leq .05$).

Table 3. Mean cumulative food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of two (2X) times normal concentration of plasma from free-feeding or 24-hour fasted birds

Treatment	Time post-injection (min) 15	30	45	60	90	120	180	240
	----------Food intake (g)----------							
Control[1]	3.1	4.4	5.3	6.0	8.9	10.4	13.3	16.3
Fed-plasma 2X	2.1	2.9*	3.4*	4.2*	5.7*	8.6	11.7	16.1
Fasted-plasma 2X	2.9	4.3	5.3	6.6	8.6	11.0	13.8	18.2
SEM[2]	.5	.4	.5	.5	.6	.6	.8	.9
	----------Water intake (ml)----------							
Control[1]	1.1	1.1	2.2	3.9	6.7	8.6	12.2	16.7
Fed-plasma 2X	1.7	2.8	3.9	5.6	8.3	10.0	14.4	21.1
Fasted-plasma 2X	1.7	3.3	5.6	6.7	10.6	11.7	15.0	18.9
SEM[2]	.6	.8	1.6	1.7	2.0	2.0	2.4	3.2

[1]Artificial cerebrospinal fluid.
[2]Standard error of the mean (n=9).
*Significantly different from control ($P \leq .05$).

Table 4. Mean cumulative food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of five (5X) times normal concentration of plasma from free-feeding or 24-hour fasted birds

Treatment	Time post-injection (min) 15	30	45	60	90	120	180
	----------Food intake (g)----------						
Control[1]	3.2	4.8	6.8	8.6	10.6	12.7	15.6
Fed-plasma 5X	2.2	3.4	4.9*	6.2*	8.1*	9.4*	13.4
Fasted-plasma 5X	3.3	4.8	6.0	7.6	8.9	10.2	14.2
SEM[2]	.5	.5	.3	.5	.7	.8	.9
	----------Water intake (ml)----------						
Control[1]	1.1	5.0	6.1	8.8	11.7	13.3	18.3
Fed-plasma 5X	.6	4.4	4.4	5.6	7.8	8.9	13.9
Fasted-plasma 5X	0.0	1.1	1.1*	3.3	5.0	6.7	11.1*
SEM[2]	.6	1.2	1.2	1.7	2.0	2.1	1.8

[1]Artificial cerebrospinal fluid.
[2]Standard error of the mean (n=9).
*Significantly different from control ($P \leq .05$).

Table 5. Mean cumulative food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of two (2X) or four (4X) times normal concentration of plasma from free-feeding or 24-hour fasted birds

Treatment	Time post-injection (min) 15	30	45	60	90	120	180	240
	----------Food intake (g)*----------							
Control[1]	3.6	5.2	6.7	7.8	9.7	11.4	15.4	17.6
Fed-plasma 2X	3.5	4.6	6.0	6.6	8.0	8.9	12.4	14.3
4X	1.9	2.7	3.7	4.9	6.2	7.2	10.4	12.4
Fasted-plasma 2X	3.8	5.3	6.4	7.5	9.1	11.1	13.6	15.2
4X	3.8	5.1	6.1	7.3	8.9	10.3	13.3	14.9
SEM[2]	.4	.5	.6	.6	.7	.7	1.1	1.2
	----------Water intake (ml)----------							
Control[1]	0.0	2.3	3.3	4.3	5.3	7.7	12.7	16.0
Fed-plasma 2X	0.0	1.3	1.3	4.3	5.0	8.3	10.0	12.3
4X	0.0	1.3	1.7	3.3	4.0	5.7	8.7	10.0
Fasted-plasma 2X	0.0	1.6	2.0	4.3	5.3	8.7	11.7	14.3
4X	0.0	1.6	2.7	4.7	5.3	7.7	11.7	15.0
SEM[2]	0.0	.9	.9	1.2	1.3	1.5	1.8	2.1

[1]Artificial cerebrospinal fluid.
[2]Standard error of the mean (n=15).
*Nonorthogonal linear contrasts were significant ($P \leq .05$) for control, 2X fed, 4X fed-plasma at all times.

Chapter 3

REDUCED FOOD INTAKE FOLLOWING INTRACEREBROVENTRICULAR ADMINISTRATION OF A LOW MOLECULAR WEIGHT FRACTION OF PLASMA FROM FREE-FEEDING FOWL

Regulation of food intake has generally been ascribed to well-defined areas within the hypothalamus. Lesioning (Brobeck et al., 1943) and anesthetizing (Epstein, 1960) the ventromedial hypothalamus (VMH) or electrically stimulating the lateral hypothalamus (LH) (Miller, 1960) increased food consumption in the rat. On the other hand, food intake was reduced in the rat by stimulating the VMH (Kranse, 1962) or by lesioning the LH (Anand and Brobeck, 1951). Similarly, hyperphagia and obesity occurred in chickens (Lepkovsky and Yasuda, 1966) and White-throated sparrows (Kuenzel and Helms, 1970) following lesioning of the VMH, whereas hypophagia and weight loss occurred in the chicken (Smith, 1969) and White-throated sparrow (Kuenzel, 1972) following lesioning of the LH.

The nature of the factor that may be acting upon the hypothalamus in vivo has not been determined, although current mammalian evidence suggests that the factor may be present in the blood. Intracerebroventricular (ICV)

injection of a narrow-spectrum molecular weight fraction of human serum into 96-hour fasted rats reduced food intake (Knoll, 1979). In the domestic fowl, food intake was reduced following ICV injection of plasma collected from free-feeding birds and concentrated to 2 or 4 times normal concentration, while injection of concentrated plasma collected from 24-hour fasted birds did not affect food intake (Chapter 2). It appears, therefore, that the hypothalamus may control food intake by monitoring the levels of various plasma components.

The objective of this study was to determine the molecular weight range of the factor(s) present in the plasma of the free-feeding domestic fowl that inhibits food intake.

MATERIALS AND METHODS

Animal preparation. Leghorn cockerels were raised in electrically heated battery brooders with food and water available ad libitum. At 8 weeks of age the birds were transferred to individual cages. Following sodium pentobarbital anesthesia (25 mg/kg), a 23 gauge stainless steel guide cannula was stereotaxically implanted into the right lateral ventricle as described by Denbow et al. (1981). The guide cannula was occluded with a 27 gauge stylet between injections. Following a 3 day recovery

period, validation of cannula location was verified by monitoring the colonic temperature response to an injection of 67 µg of norepinephrine in 10 µl of artificial cerebrospinal fluid[1] (aCSF) administered via the guide cannula. Only those birds exhibiting a decrease in body temperature of 1.0°C or greater were used in the experiments.

Plasma Preparation. Blood was collected via cardiac puncture from free-feeding and 24-hour fasted cockerels and centrifuged at 3000 g for 20 minutes. The plasma was separated into fractions using gel filtration. A 1.5 x 8 cm column (Pharmacia Fine Chemicals) containing 5 cm of Sephadex G-25 or G-15 gel (Pharmacia Fine Chemicals) was used to divide the plasma at 5000 mol wt and 1500 mol wt cut off points, respectively, yielding four fractions (<1500 mol wt, 1500-5000 mol wt, <5000 mol wt, and >5000 mol wt). Following elution with distilled water the fractions were lyophilized and reconstituted to four times the original concentration with distilled water, and stored at -20°C (appendix B).

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[1]The aCSF consisted of 155 mM Na^+, 2.5 mM Ca^{++}, 3.7 mM K^+, 2.1 mM Mg^{++}, 140 mM Cl^- and 23 mM HCO_3^- (Anderson and Hazelwood, 1969).

Injection Procedure. Injections were made via a 27 gauge stainless steel injection cannula connected to a 10 µl Hamilton syringe. In Experiments 1 and 2 the cockerels received either 10 µl of aCSF or a plasma fraction collected from free-feeding birds (see Table 1). In Experiment 3, they also received fractions of plasma collected from 24-hour fasted birds. Food and water intakes were monitored at 15-minute intervals for the first hour, 30-minute intervals for the second hour, and hourly thereafter.

Statistical design and analysis. A repeated Latin Square design (Snedecor and Cochran, 1980) was utilized. Non-orthogonal contrasts were used to make comparisons of cumulative food and water intake between the control and treated birds at each time period. Bonferroni F values ($p \leq .05$) were used in determining significance (Games, 1972).

RESULTS

Experiment 1. Injection of whole plasma collected from free-feeding birds and concentrated to four times normal significantly reduced food intake at 90 minutes post-injection (Table 2). The fraction of plasma with a molecular weight below 5000 reduced food intake at 60, 90, and 120 minutes post-injection; however, the fraction above 5000 mol wt did not alter food intake. Water intake was not significantly altered by any treatment.

Experiment 2. Food intake of the birds receiving the fraction of plasma below 1500 mol wt was less than that of the control birds at 45, 60, 90, and 180 minutes post-injection. No reduction in food intake occurred following injection with the 1500-5000 mol wt fraction (Table 3). Water intake again was not altered by any treatment.

Experiment 3. Birds receiving the below 1500 mol wt fraction of plasma collected from free-feeding birds consumed less food at 120 minutes post-injection than did the control birds, but food intake was not reduced in the birds receiving the same fraction collected from 24-hour fasted birds (Table 4). Water consumption was not significantly altered by either treatment.

DISCUSSION

Concentrated plasma fractions below 5000 mol wt and below 1500 mol wt reduced food intake if the plasma had been collected from free-feeding birds, but did not reduce food intake if the plasma had been collected from 24-hour fasted birds. The absence of a response following injection with the below 5000 mol wt fraction in Experiment 2 is to be questioned in light of the results of the other experiments in which both the <5000 mol wt and <1500 mol wt fraction reduced food intake (Experiment 1 and Experiment 3, respectively). It appears, therefore, that the "satiety"

factor previously shown to exist in the plasma of the free-feeding domestic fowl (Chapter 2), has a molecular weight below 1500.

In a similar study, a compound called "satietin", which reduced food intake when injected into the lateral ventricle of 96-hour fasted rats, was shown to exist in human serum (Knoll, 1979). Satietin was proposed to contain a limited number of peptides with molecular weights in the range of 40,000-60,000. It was reported that the proposed molecular weight range of satietin conflicted with the separation procedures employed, which included filtering all samples through an Amicon UM-10 membrane (known to withhold compounds with molecular weights above 10,000).

The lack of a response following ICV injection of plasma collected from 24-hour fasted birds was probably the result of a reduction in the level of the factor due to the nutritional status of the donors. The concentration of the factor in the plasma may decrease in proportion to the length of time the animal has gone without feeding. This type of response has been demonstrated in the rat where the amount of food consumed by a sated recipient rat following transfusion with blood from a fasted donor rat was inversely proportional to the length of deprivation of the donor rat (Davis et al., 1971).

The nature of the low-molecular weight factor(s) present in plasma that reduces food intake when injected ICV is unknown. Numerous endogenous substances with molecular weights below 1500 have been shown to decrease food intake when injected ICV. A hypophagic condition was reported in the rat (Avery and Calisher, 1982; Stuckey and Gibbs, 1982) and pig (Parrot and Baldwin, 1982) following ICV administration of bombesin. ICV injection of cholecystokinin reduced food intake in the rat (Maddison, 1977) and chicken (Denbow and Myers, 1982). Nutrient-related blood components have also been shown to be involved in regulating food intake. Infusion of 6% glucose into the lateral ventricle of domestic fowl reduced food intake for up to 3 hours post-injection (Matei-Vladescu et al., 1977).

The results of these experiments suggest that a satiety factor having a molecular weight of less than 1500 exists in the plasma of the free-feeding domestic fowl. Further, the plasma concentration of this factor appeared to be related to the nutritional status of the bird. Since water intake was not reduced by any of the treatments, it can be assumed that the effect of the treatments was specific for food intake. Whether this factor originates from the gastrointestinal tract as a secretion, e.g., cholecystokinin or bombesin, or from the absorption of nutrients, e.g., glucose, remains to be elucidated.

Table 1. Experimental outline

Experiment		
1	2	3
Control[1]	Control[1]	Control[1]
Whole plasma[2]	<5000 mol wt plasma[2]	<1500 mol wt plasma[2]
<5000 mol wt plasma[2]	5000-1500 mol wt plasma[2]	<1500 mol wt plasma[3]
>5000 mol wt plasma[2]	<1500 mol wt plasma[2]	

[1]Artificial cerebrospinal fluid.
[2]Plasma collected from free-feeding birds.
[3]Plasma collected from 24-hour fasted birds.

Table 2. Mean cumulative food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of four times normal concentration of whole, <5000 mol wt, or >5000 mol wt fractions of plasma collected from free-feeding birds

Treatment	Time post-injection (min) 15	30	45	60	90	120	180	240
	----------Food intake (g)----------							
Control[1]	3.7	4.9	6.2	7.7	10.5	11.5	16.2	18.8
Whole plasma	2.5	3.3	4.3	5.8	8.0*	10.4	14.3	18.1
>5000 mol wt plasma	3.2	4.8	5.9	6.9	9.6	11.4	15.4	18.3
<5000 mol wt plasma	2.4	3.6	4.6	5.3*	7.5*	9.0*	12.9	15.0
SEM[2]	.5	.6	.6	.5	.7	.7	.9	1.1
	----------Water intake (ml)----------							
Control[1]	1.7	2.5	3.3	5.0	7.1	11.7	19.2	24.6
Whole plasma	1.7	2.9	2.9	4.6	8.8	11.7	19.2	23.8
>5000 mol wt plasma	1.3	1.7	2.5	3.8	6.3	9.2	15.4	19.2
<5000 mol wt plasma	0.0	.4	.8	2.1	5.4	7.9	10.4	15.4
SEM[2]	1.0	1.1	1.4	1.9	2.7	2.7	2.9	3.1

[1]Artificial cerebrospinal fluid.
[2]Standard error of the mean (n=12).
*Significantly different from control ($P \leq .05$).

Table 3. Mean cumulative food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of four times normal concentration of <5000 mol wt, 1500-5000 mol wt, or <1500 mol wt fractions of plasma collected from free-feeding birds.

Treatment	Time post-injection (min) 15	30	45	60	90	120	180	240
	----------Food intake (g)----------							
Control[1]	3.4	5.3	6.5	7.2	10.5	11.9	17.1	19.8
<5000 mol wt plasma	3.8	5.5	6.7	7.2	9.8	11.7	15.6	18.7
1500-5000 mol wt plasma	2.8	4.6	5.3	6.3	8.8	10.7	14.4	18.9
<1500 mol wt plasma	2.6	3.8	4.3*	5.0*	7.0*	9.6	13.4*	16.4
SEM[2]	.4	.5	.5	.6	.6	.7	1.0	1.1
	----------Water intake (ml)----------							
Control[1]	1.7	2.9	5.0	5.4	7.5	11.3	18.3	24.2
<5000 mol wt plasma	.4	1.3	1.7	2.9	6.7	9.6	17.9	21.7
1500-5000 mol wt plasma	.4	1.7	3.8	5.4	7.5	10.8	17.9	24.6
<1500 mol wt plasma	.8	1.3	2.5	4.2	5.0	8.3	12.9	16.3
SEM[2]	.5	.8	1.1	1.1	1.5	1.9	2.6	2.7

[1]Artificial cerebrospinal fluid.
[2]Standard error of the mean (n=12).
*Significantly different from control ($P \leq .05$).

Table 4. Mean cumulative food intake of free-feeding leghorn cockerels following an intracerebroventricular injection of four times normal concentration of <1500 mol wt fractions of plasma collected from free-feeding or 24-hour fasted birds

Treatment	Time post-injection (min) 15	30	45	60	90	120	180	240
	----------Food intake (g)----------							
Control[1]	1.9	2.8	3.9	4.8	7.4	9.6	11.3	14.8
<1500 mol wt plasma[2]	1.9	2.6	3.6	4.1	5.8	6.3*	9.7	12.1
<1500 mol wt plasma[3]	1.8	2.9	4.0	4.9	7.8	9.4	11.9	14.1
SEM[4]	.4	.6	.7	.7	.7	.7	1.1	1.3
	----------Water intake (ml)----------							
Control[1]	1.1	1.7	2.8	3.3	5.6	5.6	7.8	11.7
<1500 mol wt plasma[2]	1.1	1.7	3.3	4.4	5.0	6.1	8.9	13.3
<1500 mol wt plasma[3]	0.0	.6	2.2	2.8	2.8	3.9	7.8	11.1
SEM[4]	.5	.9	1.0	1.1	1.2	1.2	2.3	2.1

[1]Artificial cerebrospinal fluid.
[2]Plasma collected from free-feeding birds.
[3]Plasma collected from 24-hour fasted birds.
[4]Standard error of the mean (n=9).
*Significantly different from control ($P \leq .05$).

Chapter 4

AVIAN CEREBROSPINAL FLUID: REPEATED COLLECTION AND TESTING FOR A POSSIBLE ROLE IN FOOD INTAKE REGULATION

A technique developed by Myers (1967) allowed cerebrospinal fluid (CSF) to be transferred between the brains of conscious monkeys, and when CSF from a food-deprived monkey was infused directly into the lateral hypothalamus (LH) of a sated monkey, food intake was stimulated (Myers, 1969). Yaksh and Myers (1972) determined that the food intake inhibiting factor was released primarily in the region of the ventromedial hypothalamus (VMH), whereas an active feeding factor was released from a less discrete area within the hypothalamus. In sheep, a five-fold increase in food intake resulted following a lateral or third ventricular injection of CSF collected from the lateral ventricle of a fasted donor (Martin et al., 1973). When CSF was collected from satiated donors and injected into the lateral ventricle of fasted sheep the result was a 20% reduction in food intake.

It has also been reported that a low molecular weight factor exists in the plasma of the domestic fowl that inhibits food intake when injected into the lateral cerebral

ventricle (Chapter 3). The possibility exists that this factor may also enter the CSF. Although it has been established that most peptides do not cross the blood-brain barrier (Conford et al., 1978), evidence exists for a limited passage of low molecular weight peptides through the blood-CSF barrier (for review see, Meisenberg and Simmons, 1983). It has also been suggested that some peptides, e.g., insulin, are selectively transported into the CSF (Pardridge, 1983). Additionally, substrates may enter the CSF by diffusing from the circumventricular organs. Once in the CSF, this factor could pass into the brain extracellular fluid.

Techniques exist for repeated collection of CSF from the rat (Pass and Ondo, 1977; Danquir et al., 1982; Kiser, 1982), monkey (Myers, 1967), sheep (Martin et al. 1973), and cat (Radulovacki, 1974). Previously, CSF has been collected from the cisterna magna of the domestic fowl by anesthetizing the bird, surgically exposing the occipital region of the skull, and inserting a 25 gauge needle through the space between the occipital protuberance and the atlas vertebra and into the cisterna magna (Anderson and Hazelwood, 1969). This technique frequently yields samples contaminated with blood and requires anesthetization of the bird.

Based on these reports the objectives of this study were to 1) develop a technique for repeated collection of blood-free CSF samples from unanesthetized birds, and 2) determine if a factor(s) exists in the CSF of the domestic fowl that alters food intake when injected into the lateral cerebral ventricle of leghorn or broiler cockerels.

MATERIALS AND METHODS

Cisterna magna cannulation of donor cockerels. The hub and shaft of a 22-gauge disposable hypodermic needle were trimmed to a length of 6 and and 14mm, respectively. The hub ridges were also removed to facilitate attachment of the protective cap which was a rubber plunger tip from a 1 cc tuberculin syringe (Figure 1).

Cannulae were stereotaxically implanted into the cisterna magna of anesthetized (sodium pentobarbital, 25 mg/kg) 10-week old broiler cockerels at the coordinates: AP=0.0, L=0.0, H=.8-1.2 mm below the skull surface (Fig. 1). A 1 mm hole was drilled in the parietal area of the skull allowing a cannula to be lowered through the cerebellum into the cisterna magna. The presence of CSF in the cannula indicated that the cisterna magna had been pervaded. Three stainless steel screws were anchored into the skull around the cannula and acrylic cement was used to fix the cannula

to the screws. Cyanoacrylate adhesive was then used to attach the rubber cap. The skin was sutured around the cannula and sprayed with an antibiotic (Topazone, Norden Laboratories, Inc.). A 7 day recovery period was provided following the surgery.

The sampling apparatus consisted of a 27 gauge withdrawal cannula attached via polyethylene tubing (PE-50,Clay Adams) to a 1 cc tuberculin syringe fitted with a 27 gauge needle. The withdrawal cannula was inserted through the rubber cap of the cisterna magna cannula for collection of CSF.

Lateral ventricular cannulation of recipient cockerels. Broiler and leghorn cockerels were raised in electrically heated battery brooders and at 4 and 8 weeks of age, respectively, transferred to individual cages (41cm x 36cm x 22.5cm). Feed and water were provided ad libitum and lighting was continuous. Following sodium pentobarbital anesthesia (25 mg/kg), a 23 gauge stainless steel guide cannula was stereotaxically implanted into the right lateral ventricle of leghorn cockerels by the procedure of Denbow et al. (1981). The guide cannula was occluded with a 27 gauge stylet between injections. Following a 3 day recovery period, validation of cannula location was determined by monitoring the colonic temperature response to an injection

of 67 µg of norepinephrine in 10 µl of artificial cerebrospinal fluid (aCSF)[1] administered via the guide cannula. Only those birds exhibiting a decrease in body temperature of 1.0°C or greater were used in the experiments.

CSF preparation and injection. Samples were collected from either free-feeding (Fed-CSF) or 24-hour fasted (Fasted-CSF) birds at 3 day intervals. The CSF was injected into free-feeding receipients at either normal concentration (1X) or was concentrated by lyophilization and reconstitution with distilled water (appendix B) to four-times its original concentration (4X) and injected (Table 1). Following a 10 µl injection, food and water intake were monitored at 15-minute intervals for the first hour, 30-minute intervals for the second hour, and hourly thereafter.

Statistical design and analysis. A repeated Latin square design was utilized (Snedecor and Cochran, 1980). Non-orthogonal contrasts were used to compare food and water intake between the control and treated birds. Bonferroni F values ($P \leq .05$) were used in determining significance (Games, 1972).

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[1] The aCSF consisted of 155 mM Na+, 2.5 mM Ca++, 3.7mM K+, 2.1 mM Mg++, 140 mM Cl-, and 23 mM HCO_3- (Anderson and Hazelwood, 1969).

RESULTS

Cisterna magna cannulation procedure. The cannulation procedure described produced clear, blood-free CSF samples of .1-.2 ml volume, every 3-days, up to 6-weeks following cannulation of broiler cockerels. This procedure was not successful in leghorn cockerels.

Broiler Recipients. The ICV injection of 1X- and 4X-CSF collected from free-feeding and 24-hour fasted donors did not affect the food intake of recipient broiler cockerels (Table 2 and 3). The ICV injection of 1X-CSF collected from free-feeding and 24-hour fasted donors did not affect the water intake of recipient broiler cockerels. Water intake, however, was significantly stimulated at 90 minutes by the 4X-CSF obtained from the fasted donors, but was not affected by the 4X-CSF obtained from the free-feeding donors.

Leghorn recipients. The ICV injection of 1X- and 4X-CSF collected from free-feeding or 24-hour fasted donors did not affect the food intake of recipient leghorn cockerels (Table 4 and 5). The ICV injection of 1X-CSF collected from 24-hour fasted donors did not alter the water intake of recipient leghorn cockerels, but when concentrated to 4X it did stimulate water intake at 90, 120, and 180 minutes

following injection. Neither the 1X- or 4X-CSF collected from free-feeding donors affected water intake.

DISCUSSION

The method described for collecting CSF from the cisterna magna allowed blood-free samples to be collected from broiler cockerels without the use of an anesthetic. Cannulae were easily fabricated from readily available inexpensive materials. Stereotaxic implanation of the cannulae required 20-30 minutes per bird. Passing cannulae through the cerebellum had no apparent affect on locomotion, balance, or coordination. Overall, the cannulation technique developed provided a relatively quick and reliable method for collecting repeated CSF samples from broiler cockerels. This procedure was not successful in leghorn cockerels, possibly due to the relatively small size of the cisterna magna.

The CSF collected from the cisterna magna did not appear to contain any factors involved in the regulation of food intake. This does not, however, eliminate the possibility of such factors existing in CSF within the lateral or third ventricles of birds, as is the case in monkeys (Myers, 1969) and sheep (Martin et al., 1973). It is possible that any active factor that may have been present in the CSF of the donor birds was metabolized or

selectively removed from the CSF prior to reaching the cisterna magna. Ashcroft et al. (1968) reported that 5-hydroxyindole acetic acid (5-HIAA), a metabolite of tryptophan, was actively removed from CSF in the fourth ventricle, thereby diminishing the level of 5-HIAA in cisternal CSF. A similar situation may have occurred in our study explaining why CSF collected from the cisterna magna did not contain a factor(s) involved in the regulation of food intake. Our efforts to collect CSF from the lateral ventricle were unsuccessful, possibly due to the relatively small size of the ventricle and the limited CSF capacity.

The reason for the stimulation of water intake following the ICV injection with fasted 4X-CSF is not known. Nevertheless, the data suggest that research to determine if an active "drinking" factor exists in CSF collected from fasted donors would be a logical subsequent study.

Table 1. Experimental outline

Experiment Number	Recipient Birds	Treatment
1	Leghorn	Control
		1X Fed-CSF[1]
		1X Fasted-CSF[2]
2	Leghorn	Control
		4X Fed-CSF[3]
		4X Fasted-CSF[4]
3	Broiler	Control
		1X Fed-CSF
		1X Fasted-CSF
4	Broiler	Control
		4X Fed-CSF
		4X Fasted-CSF

[1]Normal concentration CSF collected from free-feeding broilers.
[2]Normal concentration CSF collected from 24-hour fasted broilers.
[3]Four-times normal concentration CSF collected from free-feeding broilers.
[4]Four-times normal concectration CSF collected from 24-hour fasted broilers.

Table 2. Mean cumulative food and water intake of free-feeding broiler cockerels following an intracerebroventricular injection of normal (1X) concentration cerebrospinal fluid (CSF) collected from free-feeding (Fed-CSF) or 24-hour fasted (Fasted-CSF) birds

Treatment	Time post-injection (min) 15	30	45	60	90	120	180	240
	----------Food intake (g)----------							
Control[1]	5.0	7.9	10.7	12.9	17.3	20.3	28.7	34.2
Fed-CSF 1X	4.1	6.4	11.4	12.8	18.4	22.7	29.9	35.0
Fasted-CSF 1X	5.1	8.0	11.4	12.0	17.4	21.6	26.3	32.0
SEM[2]	.7	.9	.9	1.1	1.5	1.6	2.2	2.5
	----------Water intake (ml)----------							
Control[1]	7.2	11.1	13.9	20.0	27.8	33.9	42.8	55.6
Fed-CSF 1X	5.6	8.3	16.1	22.8	29.4	35.0	51.1	61.1
Fasted-CSF 1X	4.4	8.3	13.9	16.1	21.1	27.8	40.0	44.4
SEM[2]	1.8	2.0	3.1	4.1	3.5	4.0	4.2	3.4

[1]Artificial cerebrospinal fluid.
[2]Standard error of the mean (n=9).
*Significantly different from control ($P \leq .05$).

Table 3. Mean cumulative food and water intake of free-feeding broiler cockerels following an intracerebroventricular injection of four times normal (4X) concentration cerebrospinal fluid (CSF) collected from free-feeding (Fed-CSF) or 24-hour fasted (Fasted-CSF) birds

Treatment	Time post-injection (min) 15	30	45	60	90	120	180	240
	----------Food intake (g)----------							
Control[1]	2.3	4.8	8.1	11.1	13.9	16.9	21.9	27.8
Fed-CSF 4X	3.7	5.7	8.8	11.9	15.4	18.6	23.9	27.6
Fasted-CSF 4X	3.6	6.0	9.1	11.1	14.8	17.2	23.6	32.6
SEM[2]	.8	.8	1.2	1.5	1.6	1.5	1.8	2.1
	----------Water intake (ml)----------							
Control[1]	1.1	3.9	5.0	7.2	13.9	18.9	33.9	42.2
Fed-CSF 4X	1.1	5.0	5.6	7.8	14.4	23.3	32.8	45.0
Fasted-CSF 4X	1.1	3.9	7.8	13.3	27.8*	33.8	47.2	54.4
SEM[2]	1.0	2.1	2.9	3.1	3.8	4.9	5.8	5.9

[1]Artificial cerebrospinal fluid.
[2]Standard error of the mean (n=9).
*Significantly different from control ($P \leq .05$).

Table 4. Mean cumulative food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of normal (1X) concentration cerebrospinal fluid (CSF) collected from free-feeding (Fed-CSF) or 24-hour fasted (Fasted-CSF) birds

Treatment	Time post-injection (min) 15	30	45	60	90	120	180	240
	----------Food intake (g)----------							
Control[1]	3.6	5.0	6.3	7.7	9.9	11.4	15.7	18.6
Fed-CSF 1X	3.3	4.6	5.4	6.8	9.1	11.1	13.2	14.6
Fasted-CSF 1X	3.3	3.9	6.0	7.7	10.1	12.8	17.6	19.8
SEM[2]	.4	.3	.3	.5	.6	.9	1.3	1.4
	----------Water intake (ml)----------							
Control[1]	1.7	1.7	2.8	4.4	7.2	11.1	18.9	23.9
Fed-CSF 1X	1.7	2.2	3.9	6.1	7.8	11.1	13.3	15.0
Fasted-CSF 1X	1.7	3.3	6.7	7.2	12.2	16.1	20.6	27.2
SEM[2]	.9	1.2	1.5	1.5	2.2	2.3	3.1	3.2

[1]Artificial cerebrospinal fluid.
[2]Standard error of the mean (n=9).
*Significantly different from control ($P \leq .05$).

Table 5. Mean cumulative food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of four times normal (4X) concentration cerebrospinal fluid (CSF) collected from free-feeding (Fed-CSF) or 24-hour fasted (Fasted-CSF) birds

Treatment	Time post-injection (min) 15	30	45	60	90	120	180	240
	----------Food intake (g)----------							
Control[1]	2.7	4.3	5.8	7.8	8.8	10.9	14.9	19.0
Fed-CSF 4X	2.6	4.4	6.1	7.7	9.4	10.9	14.4	18.2
Fasted-CSF 4X	2.5	4.4	5.8	7.3	9.8	11.6	14.8	18.7
SEM[2]	.3	.4	.5	.5	.5	.6	.8	1.0
	----------Water intake (ml)----------							
Control[1]	0.6	0.6	1.1	3.3	3.9	6.7	11.1	15.6
Fed-CSF 4X	1.1	2.2	3.9	6.7	7.8	9.4	12.8	18.9
Fasted-CSF 4X	.6	1.7	4.4	6.7	11.1*	12.8*	18.3*	23.9
SEM[2]	.6	0.7	1.0	1.5	1.3	1.3	1.8	2.6

[1]Artificial cerebrospinal fluid.
[2]Standard error of the mean (n=9).
*Significantly different from control ($P \leq .05$).

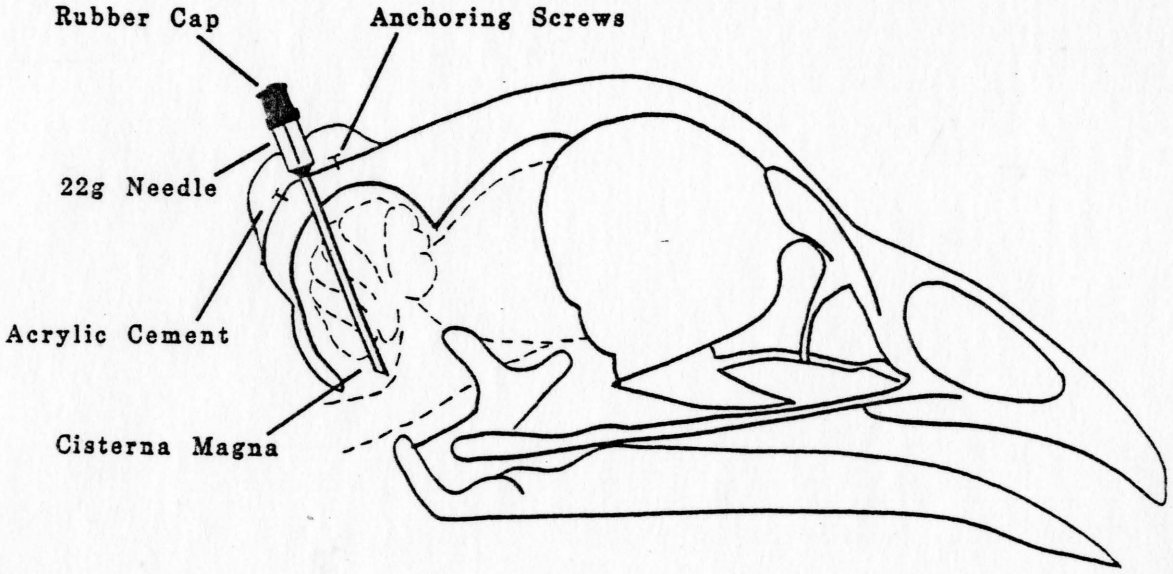

Figure 1. Illustration of CSF sampling technique and cannula construction for broiler cockerels. The brain is represented by dashed lines. (Illustration adapted from Kiser, 1982).

Chapter Five

SUMMARY AND CONCLUSIONS

Although there has been a tremendous amount of research conducted on the regulation of food intake, there is limited agreement on what type of signal is acting upon the regulatory centers within the hypothalamus. These studies, therefore, were initiated to determine if a humoral signal acting upon the hypothalamus is present in the blood or CSF of the domestic fowl.

Intracerebroventricular injection of plasma from free-feeding donors resulted in a dose dependent reduction in food intake, whereas food intake was not affected by plasma collected from fasted donors. It was subsequently established that the active factor in the plasma had a molecular weight below 1500.

The final study provided evidence that the factor present in the plasma does not exist in the cerebrospinal fluid collected from the cisterna magna. The possibility exists, however, that this factor or a similar factor may be present in the cerebrospinal fluid in other areas within the ventricular system.

The nature of this factor is still unknown. Although numerous blood-borne compounds with a molecular weight below

1500 have been shown to effect food intake, a number of peptides have received considerable attention. One of these peptides, cholecystokinin (CCK) has received more attention than others. CCK has been found in the duodenum and jejunum and its release is stimulated by the amino acid and fatty acid components of a meal (Meyer, 1974). Physiological actions of CCK include contraction of the gallbladder and stimulation of enzyme release by the pancreas (Rehfeld et al., 1980).

Centrally, CCK and CCK receptors were found in the cortex, olfactory bulbs and the hypothalamus (Saito et al., 1980). The concentration of CCK in the brain is 10 to 100 times the concentrations of other peptides (Dockray, 1980).

Peripheral administration of CCK decreased food intake in man (Sturdevant and Goetz, 1976), monkeys (Gibbs et al., 1976), mice (Koopmans et al., 1972), cats (Mendel, et al., 1980), sheep (Della-Fera and Baile, 1980), rabbits (Houpt et al., 1978) and chickens (Savory and Gentle, 1980). Central administration of CCK decreased food intake in sheep (Della-Fera and Baile, 1979) and chickens (Denbow and Myers, 1982). CCK levels have been shown to increase in both serum (Rehfeld and Krause-Larsson, 1978) and in CSF (Della-Fera et al., 1980) during feeding. Continuous central infusion of rabbit serum containing a CCK specific antibody into sheep

resulted in increased food intake (Della-Fera et al., 1981). Della-Fera et al. (1980) hypothesized that CCK may be released from specific areas of the brain in response to stimulation of the GI tract and work as a neurotransmitter, being carried by the CSF to receptor sites which when stimulated terminate feeding.

Bombesin (BN), another neuro-gut peptide, exhibits biological activity similar to CCK. BN has been detected in the GI tract and the brain (Walsh and Dockray, 1978; Brown et al., 1978). The highest concentration of BN in the brain was in the hypothalamus (Villarreal and Brown, 1978). Specific BN receptors have been reported in the hypothalamus (Pert et al., 1980) and the hippocampus (Moody et al., 1978). The effects of BN on the GI tract include stimulation of the smooth muscle which alters GI motility (Caprilli et al., 1975), stimulation of pancreatic exocrine secretion and gallbladder contraction (Basso et al., 1975) and stimulation of gastrin release (Erspamer and Melchiorri, 1973). Central administration resulted in hypothermia (Brown et al., 1979a) and hyperglycemia, hypoinsulinemia, and hyperglucagonemia (Brown et al., 1977b). Food intake was reduced by peripheral (Gibbs et al., 1979; Stein and Woods, 1981; McLaughlin and Baile, 1981) and central (Morley and Levine, 1981; Parrott and Baldwin, 1982; Stuckey and Gibbs, 1982) injection of bombesin.

Anorexigenic peptide (AXP), a tripeptide isolated from the urine of anorexia nervosa patients, has also been examined for it's effects on food intake. Systemic administration of AXP reduced food intake and lowered body weights of mice (Reichelt et al., 1978; Trygstad et al., 1978). Similar administration of synthetic AXP by others (Bauce et al., 1981; Nance et al., 1979) had no effect on food intake in either rats or mice. Intracerebroventricular administration of AXP had no effect on food intake in rats, but did cause a hypothermic condition which resulted in a reduction in body weight (Myers et al., 1983).

In addition to calcitonin's (CT) role in lowering blood calcium (Munson, 1976), it has also been shown to reduce food intake. Peripheral and central administration of CT resulted in reduced feeding in both monkeys and rats (Freed et al., 1979; Perlow et al., 1980). The actions of CT were not limited to food intake when it was administered centrally. Intracerebroventricular injection of CT reduced water intake as well as food intake (Twery et al., 1982). With a molecular weight of 4500, CT could not be responsible for the reduction of food intake found in this study, since the active factor in the blood of the domestic fowl has a molecular weight below 1500.

Although it is speculative to suggest that the unknown satiety factor is CCK, it would be a logical choice based upon previous reports. Additional evidence suggesting that CCK is the satiety factor in the Fed-plasma was provided by a preliminary HPLC peptide separation (appendix C). Using this procedure a common peak with an elution time of 145 seconds was found in the chromatograph of the Fed-plasma and in the chromatograph of Sincalide (CCK-8). Although bombesin or anorexigenic peptide could be possible alternatives, their effects on food intake are far less specific than the effects of CCK.

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APPENDIX A

Analyis of variance tables

Appendix A Table 1. Analysis of variance for food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of normal (1X) concentration plasma from free-feeding or 24-hour fasted birds

Source of variation		Time post-injection (min) 15	30	45	60	90	120	180	240
	-df-	-----Sum of squares-food intake-----							
Bird	8	23.67	8.04	31.48	34.59	63.59	59.95	99.33	155.33
Day	2	14.60	11.25	88.80	111.48	179.19	159.41	104.22	419.56
Treatment	2	12.33	8.60	15.81	17.15	20.26	32.25	14.78	13.44
Fed-plasma(1X)[1]	1	4.00	3.36	8.03	12.25	5.44	10.03	13.44	3.36
Fasted-plasma(1X)[1]	1	2.50	1.00	1.00	0.03	4.69	6.25	0.69	3.36
Error	14	82.87	120.15	205.37	210.04	327.70	433.48	621.00	661.00
	-df-	-----Sum of squares-water intake-----							
Bird	8	31.48	175.00	242.59	316.67	500.00	703.70	717.59	1148.15
Day	2	24.53	88.89	118.97	116.66	200.00	292.60	264.80	342.60
Treatment	2	6.48	11.11	6.48	11.11	8.33	12.04	48.15	48.15
Fed-plasma(1X)[1]	1	6.25	11.11	6.25	11.11	6.25	11.11	44.44	44.44
Fasted-plasma(1X)[1]	1	2.78	2.78	2.78	2.78	6.25	0.69	2.78	25.00
Error	14	139.81	375.00	512.04	688.89	775.00	1495.37	3578.70	6759.26

[1]Contrast of control vs. the treatment listed.
*Significantly different from control (P≤.05).

Appendix A Table 2. Analysis of variance for food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of two times normal (2X) concentration plasma from free-feeding or 24-hour fasted birds

Source of variation		Time post-injection (min) 15	30	45	60	90	120	180	240
	-df-	-----Sum of squares-food intake-----							
Bird	8	19.63	51.33	34.30	29.85	88.30	90.00	204.52	350.00
Day	2	24.07	11.56	9.85	22.30	35.19	38.89	31.63	338.89
Treatment	2	4.96	13.56	21.41	26.74	56.52	29.56	22.30	24.22
Fed-plasma(2X)[1]	1	4.50	10.89	16.03	14.22*	46.72*	16.06	12.50	0.22
Fasted-plasma(2X)[1]	1	0.22	0.06	0.00	1.39	0.50	1.39	0.89	16.06
Error	14	26.96	22.22	28.07	25.63	39.63	51.56	87.41	109.56
	-df-	-----Sum of squares-water intake-----							
Bird	8	40.74	51.85	83.33	212.96	590.74	557.41	366.67	816.67
Day	2	46.30	57.41	72.22	146.30	290.74	362.96	705.56	1838.89
Treatment	2	1.85	24.07	50.00	35.19	68.52	35.19	38.89	88.89
Fed-plasma(2X)[1]	1	1.39	12.50	12.50	12.50	12.50	5.56	22.22	88.89
Fasted-plasma(2X)[1]	1	1.39	22.22	50.00	34.72	68.06	34.72	34.72	22.22
Error	14	51.85	85.19	311.11	351.85	490.74	518.52	755.56	1322.22

[1]Contrast of control vs. treatment listed.

*Significantly different from control (P≤.05).

Appendix A Table 3. Analysis of variance for food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of five times normal (5X) concentration plasma from free-feeding or 24-hour fasted birds

Source of variation		Time post-injection (min)						
		15	30	45	60	90	120	180
	-df-	-----Sum of squares-food intake-----						
Bird	8	34.52	35.33	30.74	44.67	54.07	85.33	153.85
Day	2	3.63	8.00	45.41	24.67	40.52	76.22	111.19
Treatment	2	6.74	10.67	12.96	24.67	28.07	50.89	20.52
Fed-plasma(5X)[1]	1	4.50	8.00	12.50	24.50	26.89	46.72	20.06
Fasted-plasma(5X)[1]	1	0.06	0.00	1.39	4.50	12.50	26.89	8.00
Error	14	32.96	38.00	14.96	32.67	53.41	76.22	106.96
	-df-	-----Sum of squares-water intake-----						
Bird	8	16.67	240.74	233.33	185.19	307.41	379.63	600.00
Day	2	5.56	24.07	22.22	12.96	7.41	35.19	0.00
Treatment	2	5.56	79.63	116.67	140.74	201.85	207.41	238.89
Fed-plasma(5X)[1]	1	1.39	1.39	12.50	50.00	68.06	88.89	88.89
Fasted-plasma(5X)[1]	1	5.56	68.06	112.50	138.89	200.00	200.00	234.72*
Error	14	38.89	196.29	194.44	362.96	490.74	574.07	427.78

[1]Contrast of control vs. treatment listed.
*Significantly different from control (P≤.05).

Appendix A Table 4. Analysis of variance for food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of two and four times normal (2x), (4X) concentration plasma from free-feeding or 24-hour fasted birds

Source of variation		Time post-injection (min) 15	30	45	60	90	120	180	240
	-df-	-----Sum of squares-food intake-----							
Bird	14	91.55	139.79	168.35	153.28	119.95	258.19	264.74	442.35
Day	4	30.61	38.72	55.01	143.01	202.48	259.65	201.15	145.28
Treatment	4	40.08	68.99	87.15	80.08	110.35	179.39	200.21	209.28
Linear-fed	1	22.53*	45.63*	67.50*	61.63*	90.13*	132.30*	187.50*	202.80
Linear-fasted	1	0.30	0.13	2.13	1.63	4.80	8.53	32.03	53.33
Error	52	151.71	190.69	244.64	323.71	400.77	435.36	875.84	1204.24
	-df-	-----Sum of squares-water intake-----							
Bird	14	0.00	136.67	272.00	192.00	220.00	598.00	717.59	1838.67
Day	4	0.00	100.00	22.00	215.33	100.00	781.33	1354.67	888.67
Treatment	4	0.00	10.00	38.67	15.33	20.00	81.33	564.67	342.00
Linear-fed	1	0.00	7.50	20.83	7.50	13.33	30.00	120.00	270.00
Linear-fasted	1	0.00	3.33	3.33	0.83	0.00	0.00	7.50	7.50
Error	52	0.00	570.00	629.33	1179.83	1310.00	1757.33	2664.00	3319.33

[1] Linear contrast of control, 2X, 4X plasma.
*Significant nonorthogonal linear contrast (P≤.05).

Appendix A Table 5. Analysis of variance for food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of four times normal concentration of whole, <5000 mol wt, or >5000 mol wt fractions of plasma from free-feeding birds

Source of variation		Time post-injection (min) 15	30	45	60	90	120	180	240
	-df-	-----Sum of squares-food intake-----							
Bird	11	64.56	89.17	111.73	129.06	155.73	191.17	326.06	690.42
Day	3	30.73	64.50	74.06	71.23	69.06	52.67	24.34	67.42
Treatment	3	12.56	24.50	32.73	39.96	69.56	48.83	72.56	106.08
Whole plasma[1]	1	8.17	15.04	22.04	20.17	37.50	7.04	22.04	2.67
>5000 mw plasma[1]	1	1.50	0.04	0.37	3.38	5.04	0.04	3.38	1.04
<5000 mw plasma[1]	1	9.38	10.67	15.04	32.67*	54.00*	37.50*	63.38	84.38
Error	30	96.96	124.50	127.96	105.63	152.13	167.00	313.29	464.00
	-df-	-----Sum of squares-water intake-----							
Bird	11	118.23	143.75	293.23	405.73	639.75	843.23	1597.92	1355.73
Day	3	80.73	127.08	168.23	176.56	235.42	318.23	560.42	709.89
Treatment	3	22.40	43.75	43.23	59.90	72.92	126.56	618.75	655.73
Whole plasma[1]	1	0.00	1.04	1.04	1.04	16.67	0.00	0.00	4.17
>5000 mw plasma[1]	1	1.04	4.17	4.17	9.38	4.17	37.50	84.38	176.04
<5000 mw plasma[1]	1	16.67	26.04	37.50	51.04	16.07	84.38	459.38	504.17
Error	30	340.63	466.67	694.79	1069.79	2629.17	2536.46	2970.83	3378.18

[1]Contrast of control vs. treatment listed.
*Significantly different from control (P≤.05).

Appendix A Table 6. Analysis of variance for food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of four times normal concentration of <5000 mol wt, 1500-5000 mol wt, or <1500 mol wt fractions of plasma from free-feeding birds

Source of variation		Time post-injection (min)							
		15	30	45	60	90	120	180	240
	-df-	-----Sum of squares-food intake-----							
Bird	11	98.23	125.42	120.06	169.17	174.42	205.42	354.75	611.91
Day	3	11.40	27.42	19.40	25.50	22.08	59.58	45.75	31.42
Treatment	3	12.23	20.08	45.73	37.67	83.58	40.75	89.58	75.75
<5000 mw plasma[1]	1	1.04	0.38	0.67	0.00	2.67	0.38	13.50	8.17
1.5k-5k mw plasma[1]	1	2.67	2.67	8.17	4.17	16.67	9.38	42.67	5.04
<1500 mw plasma[1]	1	4.17	12.04	30.38*	28.17*	73.50*	32.67	80.67*	70.04
Error	30	68.13	73.00	93.13	107.33	109.83	174.17	337.17	446.83
	-df-	-----Sum of squares-water intake-----							
Bird	11	41.67	168.23	218.23	355.73	341.67	862.50	1043.23	1029.17
Day	3	8.33	30.73	5.73	30.73	70.83	62.50	218.23	275.00
Treatment	3	12.50	22.40	76.56	51.56	50.00	62.50	239.06	529.17
<5000 mw plasma[1]	1	9.38	16.67	66.67	37.50	4.17	16.67	1.04	37.56
1.5k-5k mw plasma[1]	1	9.38	9.38	9.38	0.00	0.00	1.04	1.04	1.04
<1500 mw plasma[1]	1	4.17	16.67	37.50	9.38	37.50	51.04	176.04	376.04
Error	30	104.17	253.13	423.96	423.96	854.17	1262.50	2473.96	2533.33

[1]Contrast of control vs. treatment listed.
*Significantly different from control (P≤.05).

Appendix A Table 7. Analysis of variance for food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of four times normal concentration of <1500 mol wt fractions of plasma collected from free-feeding or 24-hour fasted birds

Source of variation		Time post-injection (min)							
		15	30	45	60	90	120	180	240
	-df-	-----Sum of squares-food intake-----							
Bird	8	28.07	42.52	56.07	56.52	102.67	70.00	195.63	338.67
Day	2	0.52	4.74	6.74	17.85	2.00	12.67	37.85	62.00
Treatment	2	0.07	0.52	0.96	3.19	20.67	60.22	24.07	34.67
<1500 Fed-plasma[1]	1	0.00	0.22	0.50	2.00	12.50	46.72*	12.50	32.00
<1500 Fast-plasma[1]	1	0.06	0.06	0.55	0.05	0.50	0.05	1.39	2.00
Error	14	24.74	43.41	60.30	68.96	56.67	65.78	161.41	198.67
	-df-	-----Sum of squares-water intake-----							
Bird	8	35.19	62.96	66.67	124.07	100.00	157.41	390.74	546.30
Day	2	7.41	12.96	5.56	7.41	5.56	1.85	168.52	401.85
Treatment	2	7.41	7.41	5.56	12.96	38.89	24.07	7.41	24.07
<1500 Fed-plasma[1]	1	0.00	0.00	1.39	5.56	1.39	1.39	5.56	12.50
<1500 Fast-plasma[1]	1	5.56	5.56	1.39	1.39	34.72	12.50	0.00	1.39
Error	14	35.19	96.30	138.89	146.30	172.22	190.74	670.74	540.74

[1]Contrast of control vs. treatment listed.
*Significantly different from control (P≤.05)

Appendix A Table 8. Analysis of variance for food and water intake of free-feeding broiler cockerels following an intracerebroventricular injection of normal (1X) concentration cerebrospinal fluid (CSF) collected from free-feeding (Fed-CSF) or 24-hour fasted (Fasted-CSF) birds

Source of variation	Time post-injection (min)							
	15	30	45	60	90	120	180	240
	-df-	-----Sum of squares-food intake-----						
Bird	8	112.52	46.00	128.07	98.67	147.19	268.74	306.96	593.19
Day	2	85.85	81.56	197.85	240.89	285.41	457.19	626.96	592.30
Treatment	2	5.41	13.56	3.63	4.22	6.74	24.52	58.74	43.63
Fed-CSF(1X)[1]	1	3.56	9.39	2.72	0.06	5.56	24.50	6.72	2.72
Fasted-CSF(1X)[1]	1	0.06	0.05	2.72	3.56	0.06	6.72	24.50	22.22
Error	14	69.41	101.56	102.52	142.89	271.85	329.30	620.96	796.07
	-df-	-----Sum of squares-water intake-----						
Bird	8	151.85	635.19	1029.63	1446.30	1916.67	1433.33	1312.96	2196.30
Day	2	779.63	1179.63	1412.96	2229.63	2755.56	4238.89	5624.07	4068.52
Treatment	2	35.19	46.30	29.63	201.85	350.00	272.22	601.85	1296.30
Fed-CSF(1X)[1]	1	12.50	34.72	22.22	34.72	12.50	5.56	312.50	138.89
Fasted-CSF(1X)[1]	1	34.72	34.72	0.00	68.06	200.00	168.06	34.72	555.56
Error	14	418.52	524.07	1224.07	2168.52	1544.44	2022.22	2207.41	1468.42

[1]Contrast of control vs. treatment listed.
*Significantly different from control (P≤.05).

Appendix A Table 9. Analysis of variance for food and water intake of free-feeding broiler cockerels following an intracerebroventricular injection of four times normal (4X) concentration cerebrospinal fluid (CSF) collected from free-feeding (Fed-CSF) or 24-hour fasted (Fasted-CSF) birds

Source of variation		Time post-injection (min)							
		15	30	45	60	90	120	180	240
	-df-	-----Sum of squares-food intake-----							
Bird	8	14.74	41.41	84.67	342.74	290.96	317.33	596.07	475.63
Day	2	41.41	6.74	2.89	5.85	14.52	48.22	163.85	78.30
Treatment	2	9.85	7.19	4.67	8.07	10.96	14.00	21.63	143.53
Fed-CSF(4X)[1]	1	8.00	3.56	2.00	2.72	10.89	12.50	18.00	0.22
Fasted-CSF(4X)[1]	1	6.72	6.72	4.50	8.00	3.56	0.50	14.22	102.72
Error	14	72.07	79.41	177.78	269.41	333.19	265.11	393.85	554.07
	-df-	-----Sum of squares-water intake-----							
Bird	8	50.00	235.19	416.67	433.33	1712.96	2779.63	3812.96	6283.33
Day	2	38.89	7.41	72.22	238.89	257.41	279.63	1090.74	272.22
Treatment	2	0.00	7.41	38.89	205.56	1112.96	1068.52	1162.96	738.88
Fed-CSF(4X)[1]	1	0.00	5.56	1.39	1.39	1.39	88.89	5.56	34.72
Fasted-CSF(4X)[1]	1	0.00	0.00	34.72	168.06	868.06*	1012.50	800.00	672.22
Error	14	127.78	535.19	1038.89	1238.89	1846.30	3018.52	4296.30	4322.22

[1]Contrast of control vs. treatment listed.
*Significantly different from control (P≤.05).

Appendix A Table 10. Analysis of variance for food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of normal (1X) concentration cerebrospinal fluid (CSF) collected from free-feeding (Fed-CSF) or 24-hour fasted (Fasted-CSF) birds

Source of variation		Time post-injection (min)							
		15	30	45	60	90	120	180	240
	-df-	-----Sum of squares-food intake-----							
Bird	8	30.52	42.07	47.85	58.96	47.63	32.67	55.41	70.96
Day	2	10.30	14.52	8.30	11.63	0.30	6.22	37.85	39.41
Treatment	2	0.29	5.63	3.63	4.74	4.96	14.00	84.96	134.30
Fed-CSF(1X)[1]	1	0.22	0.89	3.56	3.56	2.72	0.50	26.89	72.00
Fasted-CSF(1X)[1]	1	0.22	5.56	0.50	0.00	0.22	8.00	16.06	6.72
Error	14	17.41	12.52	14.07	28.96	48.74	105.78	216.52	251.63
	-df-	-----Sum of squares-water intake-----							
Bird	8	50.00	51.85	100.00	118.52	251.85	500.00	968.52	1129.63
Day	2	5.56	57.41	205.56	201.85	357.41	238.89	557.41	585.19
Treatment	2	0.00	12.96	72.22	35.19	135.19	150.00	257.41	718.52
Fed-CSF(1X)[1]	1	0.00	1.39	5.56	12.56	1.39	0.00	138.89	355.56
Fasted-CSF(1X)[1]	1	0.00	12.50	68.06	34.72	112.50	112.50	12.50	50.00
Error	14	94.44	196.30	288.89	296.30	607.41	677.78	1185.20	1329.63

[1]Contrast of control vs. treatment listed.
*Significantly different from control (P≤.05).

Appendix A Table 11. Analysis of variance for food and water intake of free-feeding leghorn cockerels following an intracerebroventricular injection of four times normal (4X) concentration cerebrospinal fluid (CSF) collected from free-feeding (Fed-CSF) or 24-hour fasted (Fasted-CSF) birds

Source of variation		Time post-injection (min)							
		15	30	45	60	90	120	180	240
	-df-	-----Sum of squares-food intake-----							
Bird	8	7.19	15.85	28.00	36.52	34.67	70.00	86.30	139.63
Day	2	0.52	6.74	29.56	11.19	10.89	12.67	0.52	1.41
Treatment	2	0.07	0.07	0.67	0.96	4.67	2.67	0.96	2.74
Fed-CSF(4X)[1]	1	0.06	0.06	0.50	0.06	2.00	0.00	0.89	2.72
Fasted-CSF(4X)[1]	1	0.06	0.06	0.00	0.89	4.50	2.00	0.06	0.50
Error	14	14.74	21.85	32.44	33.85	37.78	47.33	81.85	122.52
	-df-	-----Sum of squares-water intake-----							
Bird	8	35.19	40.74	157.41	466.67	285.19	429.63	235.19	483.33
Day	2	1.85	24.07	12.96	16.67	46.29	35.19	96.30	238.89
Treatment	2	1.85	12.96	57.41	66.67	235.19	168.52	257.41	316.67
Fed-CSF(4X)[1]	1	1.39	12.50	34.72	50.00	61.06	34.72	12.50	50.00
Fasted-CSF(4X)[1]	1	0.00	5.60	50.00	50.00	234.72*	168.06*	234.72*	312.50
Error	14	46.30	62.96	129.63	266.67	201.85	212.96	412.96	827.78

[1]Contrast of control vs. treatment listed.
*Significantly different from control (P≤.05).

APPENDIX B

Lyophilization procedure

Lyophilization procedure

1. Plasma or CSF was stored in a lyophilization flask at -20°C until frozen.

2. The lyophilization flasks were attached to the freeze-dryer (Virtis, model 10-010), with the condenser temperature at -40°C and a vacuum of 100 microns or higher.

3. Samples were dried until there were no cold spots detectable on the outside of the flask, indicating that the residual moisture was below 1%.

4. Lyophilized plasma or CSF was then reconstituted with glass distilled water which had been filtered through a .2 micron membrane filter (Gelman Sciences, Inc.).

5. The reconstituted plasma and CSF was stored at -20°C until needed.

APPENDIX C

HPLC peptide separation procedure

HPLC peptide separation procedure

Mobile phase - acetonitrile/10% trifluoroacetic acid with 1% morpholine (34%/66%)

Column - Zorbax TMS (Dupont) 4.6mm id x 25cm

Flow rate - 2 ml/min

Detector - UV 254 nm

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Temperature - 23°C

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