

PROTOCOL FOR ANIMAL USE AND CARE*Handwritten forms are not accepted***CNPRC**

EH&S USE ONLY

**PROTOCOL #_10545_
EXPIRES: __4/10/04**

Investigator		Contact	
Last Name:		Last Name:	
First:		First:	
Middle:		Middle:	
email:		email:	
Department:		Department:	
Phone / Fax:		Phone:	
After hrs. #:		After hrs. #:	

Species (common names):	Number:	Source:
Rhesus monkey	30	CRPRC

Project Title	Effects of formula protein source on infant growth, protein and glucose metabolism		
Overnight housing location::	CRPRC	Day use only :	
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator <i>(If investigator maintained, attach husbandry SOP's.)</i>		

Procedures: Provide a one or two sentence layman's description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

The purpose of this project is to study the effect of formula protein source on protein and glucose metabolism in infants. Infant monkeys will be exclusively breast-fed or fed formula (commercially available) containing different protein sources for 4 months. Weight and length will be assessed at birth and monthly, food intake will be recorded daily and blood will be drawn at birth and bimonthly for CBC and further analysis. Glucose tolerance tests will be conducted at 1 and 3 mo.

Special Husbandry Requirements: Describe any special requirements your animals have with respect to **food, water, temperature, humidity, light cycles, caging type, bedding,** or any other conditions of husbandry.

Animals will be breast-fed, or fed experimental infant formula from birth to 4 months. No solid food will be given during this time. They will be housed in individual isolettes from birth to 1 mo and pair-caged for the duration of the study.

Other instructions for animal care staff: (check applicable entries)

Sick Animals	Dead Animals	Pest Control
<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator
<input type="checkbox"/> Clinician to treat	<input checked="" type="checkbox"/> Save for Investigator	<input type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input type="checkbox"/> Necropsy	<input checked="" type="checkbox"/> Necropsy	

Hazardous Materials *(only if in the animal room):*

Infectious Agents?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Radioisotopes?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Chemical Carcinogens?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Toxic Chemicals?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	

Funding source:	Infant formula manufacturer	Previously approved?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	NA

What Veterinarian or veterinary clinic will provide care for your animals? (check one)

<input type="checkbox"/>	Lab Animal Health Clinic (2-0514)	<input checked="" type="checkbox"/>	California Primate Research Center (2-0447)
<input type="checkbox"/>	VMTH Large Animal Field Service (2-0292)	<input type="checkbox"/>	Another Veterinarian

If you checked "Another Veterinarian", please provide:

Veterinarian:		Address:	
Day phone:			
Emergency phone:		Email:	

If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email ptillman@ucdavis.edu) for current information about training and record keeping requirements.

Summary of Procedures:

a) Briefly describe the **overall intent** of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.

Formula-fed infants consistently weigh more than breast-fed infants, suggesting specific effects of infant formula components on intermediary metabolism. Some studies have observed that formula-fed infants (3-6 mo) have enhanced insulin response to a meal compared to breast-fed infants while blood glucose levels are similar suggesting insulin resistance (an abnormally low response of the target cells to insulin) and these differences still exist at 9 mo. Previously we have observed that infant monkeys at 1 and 2 mo have higher fasting glucose and insulin than formula-fed infants; however, this difference disappears by 3 mo of age. While insulin response to a glucose load at 2 mo of age was not different between breast-fed infants and infants fed a whey-predominant infant formula, infants fed soy formula had a less robust response, suggesting that protein source may potentially play a role in modulating glucose metabolism.

Whey-predominant infant formulas have recently become more frequently used than the "classical" casein-predominant formulas. In addition, some modern whey-predominant formulas now contain novel milk protein fractions that make the whey protein composition of the formula more similar to that of human milk whey. These protein sources lead to pronounced differences in amino acid composition of the formulas, particularly in the branched-chain amino acids (BCAA), which in itself may affect insulin and glucose metabolism in infants. In a recent infant rhesus monkey study we performed, the BCAA content of the formulas did not appear to explain the pronounced differences in metabolic response to various infant formulas that we have observed. It is therefore important to further delineate the differences in formula protein composition that explain the different responses observed in insulin and glucose metabolism.

The increase in prevalence of atopic disease has encouraged the use of hydrolyzed infant formula; however, clinical studies evaluating the metabolic consequences of consumption of this type of diet have been very limited. The limited studies conducted in term infants have observed no difference in weight gain between infants fed standard infant formula and infant formula that has been extensively hydrolyzed through 4 mo of age; however, several plasma amino acids and serum albumin were lower in infants fed hydrolyzed infant formula. To our knowledge, there is little information available in human infants as to the consequences of a hydrolyzed protein diet on parameters of glucose metabolism. However, the enzymatic hydrolysis of casein may produce peptides that are similar in structure to insulin and as gut closure is latent in formula-fed infants, these caseino-peptides may be absorbed and therefore play a physiological role in modulating glucose metabolism during infancy. In a recent study we conducted in human infants, infants fed hydrolysate formula had very different plasma amino acid profiles and BUN than infants fed regular formula. However, long-term or short-term endocrine response to feeding was not studied, nor was glucose (or lactose) tolerance. We therefore find it important to study these outcomes in our infant rhesus monkey model, as we are able to draw blood samples frequently and also perform glucose tolerance tests and measure hormonal response to feeding. As a corollary, we will also assess circulating levels of liver enzymes, as these have recently been found to be significantly affected by diet, possibly reflecting early regulation (imprinting?) of liver metabolism by diet.

b) Procedures employed in this project:

Please check the appropriate boxes if any of these procedures will be employed in your project:

- | | | |
|---|---|---|
| <input type="checkbox"/> Monoclonal Antibody Production ** | <input type="checkbox"/> Food or water restriction | <input checked="" type="checkbox"/> Special diets; food or water treatment. |
| <input type="checkbox"/> Polyclonal Antibody Production ** | <input type="checkbox"/> Non-recovery surgical procedures | <input type="checkbox"/> Induced illness, intoxication, or disease |
| <input type="checkbox"/> LD 50 or ID50 studies. | <input type="checkbox"/> Survival surgical procedures | <input type="checkbox"/> Death as an endpoint (see i below) |
| <input checked="" type="checkbox"/> catheters, blood collection, intubation | <input type="checkbox"/> Multiple survival surgery | <input type="checkbox"/> Trapping, banding or marking wild animals |
| <input type="checkbox"/> Prolonged restraint. (8 hrs+) | <input type="checkbox"/> Behavioral modification. | <input type="checkbox"/> |
| <input checked="" type="checkbox"/> Fasting prior to a procedure. | <input type="checkbox"/> Aversive conditioning. | <input type="checkbox"/> |

** If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.

c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)

Newborn rhesus monkeys will be removed from their mothers and housed individually in incubators for their first month of life. Subsequently, they will be pair-caged for the duration of the study and be exclusively fed experimental infant formulas or breast-fed from birth to 4 months of age. The formulas are comprised of :

- Group 1: Whey-predominant
- Group 2: Casein-predominant
- Group 3: Whey-hydrolysate
- Group 4: Casein-hydrolysate
- Group 5: Breast-fed control

The study will begin at birth and continue until 4 months of age. Food intake will be recorded daily. All animals will be fasted for exactly 2 hours prior to blood draw. Blood from the femoral vein will be collected (1 cc into a grey-top Vacutainer; 1 cc into a heparinized Vacutainer; 2 cc into an anticoagulant-free Vacutainer, and 0.5cc into a purple microtainer) at birth and every month until 4 months of age for CBC analysis at the primate center (no FACS analysis) and our further laboratory analysis. Glucose tolerance tests will be administered at 1 and 3 mo. Infants will be fasted for exactly 2 hours and 0.5 ml of blood will be obtained in grey-top Vacutainers. Infants will be oro-gastrically intubated with glucose in water (1 mg/kg body weight) and 0.5 ml of blood will be obtained in grey-top Vacutainers 30, 60, 90 and 120 minutes after intubation.

Body weights and crown-rump measurements will be recorded monthly.

Dams of the breast fed infants will be anesthetized with ketamine, 10mg/kg IM, monthly to facilitate weighing and measuring their infants.

All blood draw volumes will comply with the CNPRC blood draw guidelines.

d) Study Groups and Numbers: Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; **you may add as many rows as you require**. The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

Group	Procedures / Drugs	Number of Animals	Category
1	Fasted, blood drawn, oral gavage (2)	6	1
2	Fasted, blood drawn, oral gavage (2)	6	1
3	Fasted, blood drawn, oral gavage (2)	6	1
4	Fasted, blood drawn, oral gavage (2)	6	1
5	Fasted, blood drawn, oral gavage (2)	6	1

Categories of invasiveness

Category	Description
1	Little or no discomfort or stress Examples: domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral.
2	Minor stress or pain of short duration Examples: cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress
3	Moderate to severe distress Examples: major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation
4	Severe pain near, at or above the pain tolerance threshold Examples: exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress; Surgical experiments which have a high degree of invasiveness.

Further descriptions of these categories are included in the instructions following this document.

e) **Rationale for species and numbers:** How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

The rhesus monkey has many similarities in milk profiles and gastrointestinal function with human infants. Additionally, the period of lactation is similar to that of humans and unlike other animal models, no special modifications of infant formula are needed in order to maintain their long-term health. This makes this model ideal for assessing the effects of dietary components on infant health.

Due to previous research in this area, we have determined that 6 animals/group gives acceptable standard deviations as a function of inter-animal variability.

f) **Surgery:** If the project involves survival surgery, where will the surgery be conducted?

Building:

Room:

Who will be the surgeon?

g) **Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:**

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

Provide the following information about any of these drugs that you intend to use in this project.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
M. mulatta	ketamine	10	IM	As needed by vet

h) **Neuromuscular blocking agents** can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:

Why do you need to use a neuromuscular blocking agent?

What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

Under what circumstances will incremental doses of anesthetics-analgesics be administered?

i) Adverse effects:

Describe any potential adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

Aspiration pneumonia for oral gastric tube feeding
 Pain due to blood draws
 Acute distress due to short-term restraint
 Hematoma due to blood draws

How will the signs listed above be ameliorated or alleviated? If signs are not to be alleviated or ameliorated by means of post-operative analgesics or other means, explain why this is necessary.

Failure to thrive will result in removal of animal from project
 Aspiration pneumonia will be treated by vet staff as needed
 Unusual pain or hematoma will be assessed and treated by vet staff as needed

Note: if any unanticipated adverse effects not described above do occur during the course of the study, a complete description of those effects and the steps taken to mitigate them must be submitted to the committee as an amendment to this protocol.

Is death an endpoint in your experimental procedure? Yes No

(Note: "Death as an endpoint" refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation). If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, describe the clinical signs which will dictate that an animal will be euthanized.

j) Literature search for alternatives and unnecessary duplication:

This section is specifically required by Federal law. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this study, or 2) there are alternative methodologies, but these are not appropriate for your particular study. "Alternative methodologies" refers to reduction, replacement, and refinement (the three R's) of animal use, not just animal replacement. You must also show that the study is not unnecessarily duplicative of other studies.

What was the date on which you conducted this search?

3/03

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

Database Name	Years Covered	Keywords / Search Strategy
Medline/Biosys	1990-2003	Formula, hydrolysate, infants, children
Pub med	1975-2003	Formula, hydrolysate, infants, children

What were your findings with respect to alternative methodologies?

Reduction: Due to our previous research in this area, we have determined that 6 animals/group will give us acceptable standard deviation.

Replacement: The rhesus monkey has many similarities in milk profile as well as gastrointestinal function with human infants. Additionally, the period of lactation is similar to that of humans. Unlike other animal models, no special modifications of infant formula are needed in order to maintain their long-term health. This makes this model ideal for assessing the effects of dietary components on human infant health without having to use human infants.

Refinement: This protocol allows us to use the fewest number of animals to determine significance of outcome variables.

Has this study been previously conducted?

Yes No

If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

NA

k) Disposition of animals: At what point in the study, if any, will the animals be euthanized?

NA

l) Methods of euthanasia: Even if your study does not involve killing the animals, you should show a method that you would use in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

Species	Method	Drug	Dose (mg/kg)	route
M. mulatta	overdose	pentobarbital	60mg /kg	IV

m) Surplus animals: What will you do with any animals not euthanized at the conclusion of the project?

Returned to the colony

Assurances for the Humane Care and Use of Vertebrate Animals:

Principal Investigator's Statement:

I have read and agree to abide by the *UC Davis Policy and Procedure Manual* section 290-30 (Animal Use and Care). This project will be conducted in accordance with the *ILAR Guide for the Care and Use of Laboratory Animals*, and the *UC Davis Animal Welfare Assurance* on file with the US Public Health Service. (These documents are available from the Campus Veterinarian and at <http://ehs.ucdavis.edu/>). I will abide by all Federal, state and local laws and regulations dealing with the use of animals in research.

I will advise the Animal Use and Care Administrative Advisory Committee in writing of any significant changes in the procedures or personnel involved in this project.

_____ <i>Principal Investigator</i>	_____ <i>Rank / Title</i>	_____ <i>Date</i>
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Committee Use Only Below

** Conditions necessary for Committee Approval:
Final Disposition of this protocol: <input type="checkbox"/> Approved <input type="checkbox"/> Not Approved <input type="checkbox"/> Withdrawn by Investigator Date of Action: ____/____/____

I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.

_____ <i>Campus Veterinarian</i>	_____ <i>Date</i>
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