# DISTRIBUTION OF 1-AMINOCYCLOPENTANECARBOXYLIC ACID IN THE RHESUS MONKEY*

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AMINOCYCLOPENTANE CARBOXYLIC ACID (ACPC) is an unnatural amino acid that has been found to possess antitumor activity in animals [12, 8]. Following injection it appears to be metabolically inert and not to interfere with normal tissue respiration. It is widely distributed in both cellular fluids and tissues but is not incorporated into proteins and appears to remain intact within the cell [2, 3, 6]. For this reason it has been used as a model of amino acid transport and has been found to be similar in its behavior to L-valine and L-leucine [6, 1].

ACPC which lacks the α-hydrogen of the natural metabolizable amino acids may act as an amino acid antagonist. This is suggested by the observation that the toxicity of ACPC in chickens can be alleviated by dietary supplementation with valine [10]. ACPC also inhibits the incorporation of valine into rat liver [4]. However, Mickelson [11] failed to observe any interference by ACPC with utilization of natural amino acids by certain bacteria.

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In mice, autoradiographic studies have demonstrated a selective concentration of ACPC in pancreas and bone marrow [5]. This observation suggested the possible use of ACPC in the diagnosis and treatment of pancreatic disease. Pursuing this thought, Sherman et al. [13] studied the tissue distribution of ACPC in mice, dogs, and rabbits. While confirming the selective localization of ACPC in the mouse pancreas, they were unable to demonstrate localization in the pancreas of dogs and rabbits. They ascribed the observed difference to species differences in transport systems and wondered whether metabolic pathways in the human would more closely resemble those seen in the mouse or in the dog.

Christensen and Clifford [7] noted that the urinary excretion of ACPC in humans was distinctly different from that seen in rats. It therefore seemed appropriate to study the tissue distribution of ACPC in primates.

## *Experimental Procedures*

Nine Rhesus monkeys weighing between 3.0 and 4.0 kg. were used. Carboxyl-$^{14}$C ACPC was obtained from the New England Nuclear Corporation. The specific activity was 3.8 mCi./mmole. The ACPC was dissolved in 200 ml. of sterile lactated Ringers solution at a concentration of 1 μCi./ml.

The monkeys were anesthetized with phencyclidine hydrocloride and a celiotomy

*Table 1. Mean Values and Standard Deviations (in parentheses) for the Distribution of ACPC in Rhesus Monkeys after Intravenous Injection of μCi./kg.*

| Time | Blood[a] | Muscle[b] | Pancreas[b] | Liver[b] | Kidney[b] | Spleen[b] | Stomach[b] | Small Bowel[b] |
|---|---|---|---|---|---|---|---|---|
| 15 min. | 3450 (350) | 2560 (100) | 7480 (1286) | 27,730 (8488) | 5440 (909) | | | |
| 30 min. | 2730 (320) | 2010 (244) | 6230 (1120) | 23,500 (7298) | 4415 (335) | 1360 | 2980 | 2630 |
| 60 min. | 2410 (232) | 2710 (334) | 5210 (1074) | 25,930 (7708) | 3650 (966) | | | |
| 2½ hrs. | 1670 ( 74) | 2180 (165) | 3040 ( 176) | 11,460 ( 260) | 2440 (440) | | | |
| 24 hrs. | 1080 (121) | 970 (205) | 920 ( 96) | 1,410 ( 380) | 320 ( 79) | | | |

[a]Units for blood values: counts per min./ml. whole blood.
[b]Units for tissues: counts per min./gm. wet weight of tissues.

*Table 2. Mean Values and Standard Deviations (in parentheses) for the Distribution of ACPC Following Intravenous Injection of 2 μCi./kg. with Secretin Stimulation of 2 units/kg.*

| Time | Blood[a] | Pancreas[b] | Liver[b] | Pancreatic Juice[a] |
|---|---|---|---|---|
| 15 min. | 3510 (536) | 10,170 (1840) | 11,430 (3863) | 640 (54) |
| 30 min. | 2720 (661) | 6,730 (2081) | 14,350 (4241) | 146 (46) |

[a]Units for blood and pancreatic juice: counts per min./ml. of whole blood or pancreatic juice.
[b]Units for pancreas and liver: counts per min./gm. wet weight of tissue.

*Table 3. Mean Values and Standard Deviations (in parentheses) for the Distribution of ACPC Following Intravenous Injection of 2 μCi./kg. with Pancreozymin Stimulation of 2 Units/kg.*

| Time | Blood[a] | Pancreas[b] | Liver[b] | Pancreatic Juice[a] |
|---|---|---|---|---|
| 15 min. | 3640 (555) | 9350 ( 813) | 13,540 (3808) | 740 (96) |
| 30 min. | 3070 (889) | 5870 (1216) | 13,690 (4175) | 94 (56) |

[a]Units for blood and pancreatic juice: counts per min./ml. of whole blood or pancreatic juice.
[b]Units for pancreas and liver: counts per min./gm. wet weight of tissue.

was performed. Two microcurie/kg. of ACPC was injected into the inferior vena cava and tissue specimens and blood were obtained at the appropriate intervals.

In four monkeys the pancreatic duct was cannulated and pancreatic juice was collected following stimulation with secretin in two monkeys and with pancreozymin in two monkeys. The dose of secretin and pancreozymin was 2 units/kg. given as a single intravenous injection administered simultaneously with the ACPC.

Blood and tissue specimens were prepared for liquid scintillation counting using the procedure essentially as described by Christensen and Jones [6]. Samples were counted in a Packard liquid scintillation spectrometer, Model 314X, at settings which gave a $^{14}C$-efficiency of about 35%.

## RESULTS

The tissue distribution of ACPC in monkeys following intravenous injection is summarized in Table 1. There is an initial high concentration in the liver which declines to blood levels at the end of 24 hours. At early time intervals, the concentration of ACPC in the pancreas is elevated above blood levels, but does not approach the amounts present in the liver. Other tissue concentrations are comparable to blood levels. ACPC is uniformly present in all tissues studied at 24 hours. Following stimulation with secretin and pancreozymin (Tables 2 and 3) there appears to be no significant difference in the concentration of ACPC in the pancreas or liver compared to controls. There is no significant excretion in pancreatic juice.

## DISCUSSION

The distribution of ACPC in Rhesus monkeys appears to be different from that observed in mice, rabbits, and dogs as reported by Berlinquet et al. [5] and Sherman et al. [13]. In monkeys there appears to be a significant concentration in the liver with somewhat less present in the pancreas and almost none in pancreatic juice. High concentrations of ACPC in mouse liver were noted by Christensen and Jones [6]. They observed ACPC concentrations in the liver that were 4.5 times as great as in plasma at 6 hours in the mouse, whereas concentrations in muscle were essentially the same as in plasma. Our results with monkeys are similar.

The high concentration in liver decreases

in time, and at 24 hours there is no selective concentration by any organ. The failure of others to observe high concentrations of ACPC in liver may have resulted from their not obtaining samples at time intervals less than 1–2 hours.

Large doses of ACPC (375 mg./kg.) cause significant atrophy of the pancreas in the rat with loss in pancreas weight of up to 37%. There is a decrease in the size of the acini, disappearance of zymogen granules, and a loss of enzyme activity. However, islets are preserved as is glucose tolerance [9]. Further studies to explore this observation in monkeys are planned.

## SUMMARY

The distribution of ACPC in Rhesus monkeys appears to be different from that observed in other species. High concentrations are noted in the liver following intravenous injection. ACPC is concentrated in the pancreas to a lesser degree. There is no significant excretion in pancreatic juice. Pancreatic stimulation causes no significant change in the distribution of ACPC.

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