1965 #16

DISTRIBUTION OF CARNITINE, ACETYLCARNITINE AND CARNITINE ACETYLTRANSFERASE IN SPERMATOZOA FROM VARIOUS SPECIES

I. B. Fritz and N. R. Marquis, University of Michigan, Ann Arbor, Mich.

Carnitine acetyltransferase catalyzes the reaction:

$$\text{Acetylcarnitine} + \text{CoA} \rightleftharpoons \text{acetyl CoA} + \text{carnitine}$$

Distribution of components of this reaction in various rat tissues has been investigated in detail (J. Biol. Chem., 240: 2193, 1965). Highest enzyme levels were found in spermatozoa and in the caudal epididymus from adult animals, and very high carnitine concentrations were present in reproductive tissues. The absolute in vivo dependence of enzyme system activity in reproductive tissues of the male rat upon testosterone maintenance (J. Biol. Chem., 240: 2197, 1965) led us to believe that this system may be of general importance in spermatogenesis. Along with direct approaches being attempted in an investigation of spermatogenesis in the rat, we thought it desirable to explore distribution of the components of the enzyme system in spermatocytes, and/or testes from ripe marine organisms which could be readily collected at MDIBL. During August, 1965, these included Squalus acanthias, Pecten magellanicus, Echinarachnius parma and Hydractinia echinata. In addition, eggs were collected from several species for comparison. Samples were transported back to the University of Michigan in the frozen state for analysis, and only preliminary results have thus far been obtained. Scallop testis contained .035 μmoles carnitine per g dry weight while the ovary had 0.38 μmoles. Detectable carnitine was not present in sand dollar sperm and Hydractinia gonophores. It is our intention to analyze all specimens collected for content of carnitine and acetylcarnitine, and for levels of carnitine acetyltransferase as well as carnitine palmityltransferase activity. After this survey is completed, we intend to explore in detail the metabolism of spermatozoa from one of these species in an effort to determine what portion of total energy expenditure is derived from fatty acid metabolism and what role if any the carnitine acyltransferase system plays in sperm metabolism. The apparent absence of the enzyme system from sand dollar sperm would suggest that carnitine does not play an integral portion in spermatocyte metabolism generally, and it will be of obvious importance to attempt to compare the above parameters with oxidative mitochondrial activity in sperm from various species.

1965 #17

THE RESPONSE OF BLOOD GLUCOSE AND LACTATE TO CATECHOLAMINES IN THE SKATE, Raja erinacea*

W. C. Grant, Jr. and F. J. Hendler, Williams College, Williamstown, Mass.

Mammalian insulin and glucagon produce a slight effect on blood sugar levels in the little skate, Raja erinacea. Handling and hourly removal of blood samples (0.10-0.60 ml/hr) produce

*Investigation supported by PHS grant HD01164-08.

little variation in glucose concentrations. Heat stress produces hyperglycemia while pancreatectomy has a prolonged hypoglycemic effect. In the present investigations, adult skates (0.7-1.0 kg body weight) were injected via the caudal circulation at various dose levels with crystalline norepinephrine bitartrate (Sigma lot 358-1280) and epinephrine bitartrate (Sigma lot 1038-1940) in 1.0 ml eslasmobranch Ringer's. Blood glucose levels were determined in mg% with the glucose oxidase method and mg% blood lactate was analyzed by measuring the amount of NAD.H formed after addition of LDH (lactic dehydrogenase) to blood filtrates.

Animals which received injections of 50 μg/kg norepinephrine showed a rapid rise in mg% glucose with a two hour mean peak of 75.8% above the base levels. This fell to 43.2% in 5 hours and approached base level by 24 hours. Mean lactate levels rose to 730% base level in 2 hours and dropped below the base in 24 hours, The highest values recorded for glucose and lactate were 55.1 mg% and 39.1 mg% respectively. Norepinephrine at 10 μg/kg produced erratic responses. In one case there was a 65% rise in blood glucose within 30 minutes while another animal showed no change from control levels in 24 hours. Administration of 500 μg/kg produced a rise in mg% glucose which was lower than values for animals receiving 50 μg/kg but more prolonged.

Six skates receiving 50 μg/kg epinephrine showed a change in blood sugar levels of only 24.6% (58.6 mg%) at 2 hours which was followed by a drop to 2.7% below base level in 24 hours. A rise in lactate levels of 243.0% (33.0 mg%) at 24 hours was followed by a drop of 188.0% at 48 hours. Three animals receiving 10 μg/kg epinephrine gave an almost identical response in glucose levels to skates given the 50 μg dose. However, 100 μg/kg epinephrine given to several animals produced a rapid rise of 29.0% in 30 minutes followed by a sharp decline in 3 hours below base levels. In most cases test animals showed considerable bleaching after injection of either of the catecholamines used above.

In the skate norepinephrine at 50 μg/kg (approx. concentration of 1 μg/ml plasma) had twice the hyperglycemic effect of epinephrine. This is quite different from most animals in which norepinephrine has little or no effect on blood sugar but is consistent with the fact that in elasmobranch chromaffin tissue 60-80% of the catecholamines is norepinephrine (von Euler and Fange, 1960). As in mammals, the rise in lactic acid parallels that of glucose. The fact that epinephrine caused a more prolonged elevation of lactate levels but less hyperglycemia than norepinephrine is difficult to interpret. The synergistic action between chromaffin-interrenal tissue and the hypophysis and the resulting effect upon various target organs is extremely complex. At higher doses norepinephrine (500 μg/kg) and epinephrine (100 μg/kg) were less effective hyperglycemic agents. When several skates were given doses of 1 M glutamate (730 mg/kg) and 0.1 M alanine (450 mg/kg) no changes in blood sugar levels were recorded in 24 hours and forced feeding of crab and shrimp meat also failed to produce significant change. These results support the results or earlier experiments on skates which suggest there is little mobility of blood glucose in these animals.