for adult hen heart enzyme has a distinctly arc-shaped profile, which means that deaminase from this tissue is much more affected by the pH under these conditions.

The ionization constant values of the substrate (pK for AMP: 6.1-6.4) and the activator (pK for ATP: 6.5-6.9) lie in the region of the changes observed. Thus the bend at the acidic side of the pH scale (fig.) may correspond to the pK for the free enzyme. The $pK_{0.5}$ plot concaved downward at the neutral pH agrees well with the discontinuity of the curve in the log V_{max} plot and probably exhibits the change in the ionization state of the enzyme-substrate complex.

It has been shown previously[9], that the presence of ATP (even at low concentration) transforms the sigmoid-shaped kinetics of the chicken heart AMP-deaminase into a hyperbolic one. It is evident from the experimental data obtained in this work that in the presence of 1 mM ATP both developmental forms of the heart enzyme showed hyperbolic kinetics throughout the whole pH-range tested. Thus the half-saturation constant ($K_{0.5}$) has the significance of the Michaelis constant (K_m) in these conditions.

The most interesting feature of the data presented in this paper seems to be the fact that the maximum velocity of the reaction, but not the half-saturation constant was practically pH-independent over the whole pH-range tested. This suggests that the chicken heart AMP-deaminase is influenced by hydrogen ions according to the unireactant model described by Michaelis and Davidsohn[17]:

$$\begin{array}{ccccccc} & & E & & & & E \\ K_1 & & \Updownarrow & & & & \Updownarrow \quad K_i \\ & EH + S & & \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} & EHS & \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} & EH + P \\ K_2 & & \Updownarrow & & & & \Updownarrow \quad K_2 \\ & & EH_2 & & & & EH_2 \end{array}$$

where K_1 and K_2 are the equilibrium constants referring to the first and the second stages of ionization of the enzyme molecule.

The rate equation for this model has the form:

$$v_0 = \frac{V_{max}}{1 + \frac{K_m}{[S]}\left(1 + \frac{[H]}{K_1} + \frac{[H]}{K_2}\right)}$$

where $V_{max} = k_{+2}[E_0]$.

It is noticeable from this equation that the expression for K_m is pH-dependent, but that for V_{max} it is not.

The above comparison of the effect of pH on the reaction catalyzed by adenylate deaminase from the heart of 14-day chicken embryo and of adult hen confirms the notion[9] that also in this respect the enzyme is not the same at these 2 ontogenetic stages.

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Hypolipidemic effects of garlic oil in rats fed ethanol and a high lipid diet

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Summary. Feeding of ethanol and a high fat-high cholesterol diet to rats markedly increased the total lipids in the liver, and cholesterol and triglyceride levels in the serum, liver and kidneys. However, when ethanol mixed with 0.5% garlic oil was fed to animals maintained on the high fat-high cholesterol diet, these lipid levels were significantly reduced to levels near to those seen in untreated control rats. Garlic oil did not reduce the serum albumin or the total proteins of liver, kidneys or serum when fed along with ethanol. Probably the garlic oil enhances the catabolism of dietary cholesterol and fatty acids.

Ethanol administration is known to enhance the endogenous syntheses of cholesterol[2] and fatty acids[3-5], which accumulate in the liver. Ethanol also decreases the catabolism of dietary cholesterol[6,7] and fatty acids[8,9]. Alcoholic hyperlipemia and steatosis are potentiated by a high lipid diet[5,10,11].

Massive supplementation of the diet with choline in human volunteers[12] or experimental animals[13,14] failed to prevent the alcohol-induced fatty liver. High protein diet[15] or a diet containing a complete mixture of amino acids or casein, methionine and cysteine also was ineffective in preventing alcoholic fatty liver[16-18]. Garlic oil is a potent hypolipidemic agent in sucrose-fed animals[19] and in animals fed ethanol and a low lipid diet[20].

Garlic extracts fed to normal rats were found to depress the lipid levels in liver and blood[21,22]. Hence the present work

Effects of garlic oil in rats fed ethanol and high lipid diet

	Group I Untreated control rats (6)	Group II Rats fed a high lipid diet (5)	Group III Rats fed ethanol + high lipid diet (6)	Group IV Rats fed ethanol, garlic oil and high lipid diet (5)
Serum total cholesterol (mmol/l)	3.57 ± 0.31	6.99 ± 0.916[c]	8.47 ± 0.723	3.85 ± 0.288[c]
Serum triglycerides (mmol/l)	0.821 ± 0.24	1.474 ± 0.243[b]	2.324 ± 0.418[a]	0.891 ± 0.061[c]
Serum albumin (g/l)	56.5 ± 1.2	41.9 ± 1.8[a]	37.2 ± 3.69	40.3 ± 1.56
Total serum proteins (g/l)	78.0 ± 4.0	74.0 ± 1.6	78.3 ± 3.55	74.0 ± 1.84
Liver total cholesterol (mmol/kg)	0.357 ± 0.078	1.69 ± 0.101[c]	1.96 ± 0.116[c]	0.398 ± 0.088[c]
Liver triglycerides (mmol/kg)	6.84 ± 1.03	12.0 ± 2.73[a]	17.36 ± 2.85	8.0 ± 1.16[c]
Liver total lipids (g/kg)	31.8 ± 5.6	63.6 ± 11.2[c]	84.0 ± 19.2	47.4 ± 7.8[b]
Liver homogenate proteins (g/kg)	188 ± 3.0	190 ± 4.5	230 ± 20	199 ± 4.38
Kidney total cholesterol (mmol/kg)	0.267 ± 0.052	0.554 ± 0.0176[c]	0.683 ± 0.0416[c]	0.372 ± 0.058[c]
Kidney triglycerides (mmol/kg)	3.87 ± 0.645	8.61 ± 1.75[c]	11.23 ± 0.435[a]	3.82 ± 0.537[c]
Kidney homogenate proteins (g/kg)	182 ± 6.0	178 ± 5.0	173 ± 6.3	161 ± 3.7
Change in body weight	+ 54 ± 5.0	+ 17 ± 3.5[c]	− 9 ± 4.1	+ 11 ± 5.2[c]

Results are expressed as meam ± SD, with number of animals in parentheses. Student's t-test. Significant changes are shown [a] $p < 0.05$; [b] $p < 0.02$; [c] $p < 0.01$.

was undertaken to study the hypolipidemic effects of garlic oil in animals fed ethanol and a high lipid diet.

Materials and methods. Male albino rats obtained from the Faculty of Agriculture of the University of Maiduguri, weighing 100–150 g, were used for the experiments. They were divided into 4 groups. Group I, rats fed with a standard rat diet (Pfizer, Kaduna) ad libitum, served as untreated control rats. Rats of groups II, III and IV were fed ad libitum a diet containing 59% carbohydrate, 16% protein, 20% margarine, 2% cholesterol, 2% minerals and 1% vitamin supplements. Group III rats were given 3 ml 30% ethanol daily intragastrically. Group IV rats were given the same dose of ethanol containing 0.5% garlic oil (0.15 g/kg b.wt).

The dose of garlic oil used in this experiment was the same as those used for the optimum biological effects in normal rats by earlier workers[22,23]. After 30 days of experimental feeding all the rats were sacrificed in the fed state. Groups III and IV rats were sacrificed 4 h after the last dose of ethanol. Total proteins and albumin were estimated from the serum by the method of Reinhold[24]. Proteins in the liver and kidneys were estimated by the method of Lowry et al.[25] from 10% homogenates. Total lipids in the liver and kidneys were extracted by the method of Koch-Weser et al.[26] and were estimated by weighing. Triglycerides in the serum and in the extracts of liver and kidneys were estimated by the combined method of West and Rapport[27] and Lambert and Neisch[28], after removing the phospholipids by adsorption over florisil. Total cholesterol in the serum and in the extracts of liver and kidneys were estimated by the method of Zlatkis et al.[29]. Garlic oil was extracted from fresh garlic by the method of Platenius[30]. Statistical analyses of the results were made according to Student's t-test.

Results and discussion. Feeding the rats a diet rich in fat and cholesterol (group II) resulted in a significant ($p < 0.01$) increase of total cholesterol by 95% in the serum, 373% in the liver and 107% in the kidneys, compared to untreated control rats (group I). As a result of feeding ethanol to the animals fed a high lipid diet (group III) the total cholesterol levels further increased significantly ($p < 0.01$) by 16% in the liver and 23% in the kidneys. Enhanced cholesterol accumulation in the liver when ethanol was administered with a cholesterol-containing diet has been reported by earlier workers[6]. This effect is probably due to decreased cholesterol catabolism after ethanol feeding[6,7]. However, feeding of garlic oil along with ethanol (group IV) reduced the total cholesterol levels significantly ($p < 0.01$), by 58% in the serum, 80% in the liver and 46% in the kidneys. Thus garlic oil is able to reduce the cholesterol levels in group IV rats to levels near to those in untreated control rats (group I).

Similarly the triglycerides increased in the serum by 80% ($p < 0.02$), in the liver by 86% ($p < 0.05$) and in the kidneys by 135% ($p < 0.01$) after feeding a high fat-high cholesterol diet (group II compared to untreated control rats, group I). As a result of feeding ethanol (group III) the triglycerides showed a further rise by 58% ($p < 0.05$) in the serum and 30% ($p < 0.05$) in the kidneys. Feeding of garlic oil along with ethanol and the high lipid diet (group IV) reduced the triglycerides significantly ($p < 0.01$), in the serum by 61%, in the liver by 54% and in the kidneys by 66%. Ethanol decreases the hepatic oxidation of fatty acids[8], and the fatty acids which accumulate in the liver of the animals fed ethanol and a high lipid diet are derived primarily from dietary fatty acids[3,5]. Probably the garlic oil enhanced the oxidation of dietary fatty acids in ethanol-fed animals (group IV) resulting in the reduction of triglycerides in the blood, liver and kidneys.

Total lipid in the liver was increased by 100% ($p < 0.01$) on feeding a high lipid diet (group II). Alcohol feeding did not significantly increase the total lipids in the liver ($p > 0.1$) (group III). Feeding of garlic oil and ethanol (group IV) reduced the total lipids in the liver significantly ($p < 0.02$) by 44% compared to ethanol and high lipid diet-fed rats (group III).

High lipid diet reduces the serum albumin by 26% ($p < 0.01$) (group II) compared to untreated control rats (group I). However ethanol or ethanol and garlic oil (groups III and IV) did not change the serum albumin significantly. Total protein of the serum, liver or kidneys was not affected by high lipid diet or by the addition of ethanol and garlic oil to that diet. Garlic oil is known to reduce the homogenate proteins in liver and kidneys[19], but feeding of ethanol prevents the garlic oil-induced reduction in liver and kidney proteins[20].

Gain in body weight in animals fed the high lipid diet during the period of the experiment was only 69% of the gain seen in the normal rats ($p < 0.001$). However, rats fed ethanol and the high lipid diet (group III) did not show any significant change in body weight during this period. When garlic was fed along with ethanol (group IV) there was a marginal increase in body weight compared to alcohol-fed rats (group III) ($p < 0.01$).

We conclude that garlic oil is able to reduce the hyperlipidemic effects of ethanol and a high lipid diet, probably by increasing the endogenous metabolism of fatty acids and cholesterol.

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Dipeptidyl aminopeptidase in neonatal rat brain regions

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Summary. Changes in the activity of dipeptidyl aminopeptidase in rat pituitary gland and various brain regions were examined at 3, 5 and 20 weeks of age. The enzyme activity per g tissue wet weight in pituitary gland was the highest of all tissues studied. Subcellular distribution of the activity was also studied. The highest enzyme activity was found in a crude mitochondrial fraction including synaptosomes.

Dipeptidyl aminopeptidase belongs to the group of intracellular enzymes that are capable of cleaving dipeptide moieties from the unsubstituted NH_2 termini of peptides and dipeptidyl arylamides. Developmental changes in dipeptidyl peptidase I and III activities during maturation of rat brain have been studied by Marks et al.[1]. We have also found dipeptidyl aminopeptidase activity in developing rat brain by using 7-(Gly-Pro)-4-methylcoumarinamide (Gly-Pro-MCA) as substrate[2]. Although dipeptidyl aminopeptidase II has an optimum pH between 5.0 and 6.0[3], similar to that of rat brain dipeptidyl aminopeptidase, properties of both enzymes are slightly different from each other[2]. In a previous report[2], we found that the level of dipeptidyl aminopeptidase in the young whole rat brain was higher than that in the adult rat brain. However, the real physiological role of this enzyme is little known. In this paper, we describe regional and subcellular distributions of the enzyme in developing rat brain in order to ascertain its physiological role.

Materials and methods. Timed pregnant Sprague-Dawley rats were obtained from Charles River Laboratories in Japan. Each animal was killed by decapitation. Brain tissues were quickly separated and homogenized in 9 volumes of 0.32 M sucrose. Subcellular fractions were prepared according to the method of Gray and Whittaker[4]. Enzyme activity was assayed with Gly-Pro-MCA as reported previously[5], but using Britton-Robinson's universal buffer, pH 6.0. Incubation mixture (total volume 100 µl) contained 20 µl of 1 mM Gly-Pro-MCA, 20 µl of Britton-Robinson's universal buffer (pH 6.0) and enzyme. Incubation was performed at 37 °C for 5 min and the reaction was stopped by adding 1 ml of 1 M acetate buffer, pH 4.2. 7-amino-4-methylcoumarin (AMC) liberated from Gly-Pro-MCA was determined by the fluorescence intensity at 460 nm with excitation at 380 nm.

1 unit (U) of enzyme was defined as the amount of enzyme which released 1 µmol of AMC per min from the Gly-Pro-MCA.

Results and discussion. As shown in table 1, the activity of dipeptidyl aminopeptidase in pituitary gland was 2–5 times higher than that in the other brain regions studied. The

Table 1. Regional distribution of dipeptidyl aminopeptidase activity in rat brain and pituitary gland

Tissue	Enzyme activity (U/g tissue)		
	3 weeks	5 weeks	20 weeks
Cerebellum	0.585 ± 0.062	0.560 ± 0.039	0.272 ± 0.036
Cerebrum	0.405 ± 0.103	0.344 ± 0.029	0.251 ± 0.058
Colliculi	0.421 ± 0.081	0.407 ± 0.031	0.282 ± 0.054
Pons medulla	0.611 ± 0.171	0.468 ± 0.018	0.263 ± 0.019
Midbrain	0.488 ± 0.111	0.391 ± 0.049	0.231 ± 0.007
Hypothalamus	0.415 ± 0.104	0.326 ± 0.078	0.276 ± 0.024
Pituitary gland	1.084 ± 0.027	1.333 ± 0.098	1.234 ± 0.115

Each value represents mean ± SEM, n = 3.