ers(2,3) remains to be determined. As pointed out earlier by several authors(2,3), posterior lobe powders probably constitute a poor starting material for concentration of CRF since they are maximally contaminated with ACTH, and vasopressin. Both of these substances interfere with CRF assays and vasopressin has intrinsic CRF activity(3).

Summary. Acetone powders were prepared from large numbers of ovine SME fragments. After preliminary extraction of the powder with glacial acetic acid and lyophilization, the resultant powder was extracted with 0.1 M ammonium acetate and subjected to gel filtration on a long column of Sephadex G-25. A wide zone was eluted from the column which induced adrenal ascorbic acid depletion upon its iv administration into rats with median eminence lesions. Part of this activity was caused by ACTH-like substances, since it was still present in hypophysectomized rats; however, this active zone was free of pressor activity. The active zones from several fractionations with Sephadex were pooled and placed on a CMC column. Elution was accomplished by application of gradients of ammonium acetate buffer of increasing ionic strength and pH. A narrow zone was eluted from the column which was active in inducing adrenal ascorbic acid depletion in rats with lesions, but was inactive in hypophysectomized rats. A dose of < 3 μg of peptide had significant activity. This method appears applicable for preparation of highly purified hypothalamic CRF. In this study we were able to locate only one CRF; however, the possible existence of other CRF's is not excluded.

1. Saffran, M., Schally, A. V., Can. J. Biochem. and Physiol., 1955, v33, 408.

2. Guillemin, R., Rec. Prog. Hor. Res., 1964, v20, 131.

3. McCann, S. M., Dhariwal, A. P. S., in Neuroendocrinology, vol. I, ed. L. Martini, W. F. Ganong, Academic Press, in press.

4. Ramirez, V. D., Nallar, R., McCann, S. M., Proc. Soc. Exp. Biol. and Med., 1964, v115, 1072.

5. Dhariwal, A. P. S., Antunes-Rodrigues, J., McCann, S. M., ibid., 1965, v118, 999.

6. Dhariwal, A. P. S., Nallar, R., Batt, M., McCann, S. M., Endocrinology, 1965, v76, 290.

7. Dhariwal, A. P. S., Krulich, L., Katz, S., McCann, S. M., ibid., in press.

8. Lowry, O. H., Rosebrough, N. J., Farr, A. S., Randall, R. J., J. Biol. Chem., 1951, v193, 265.

9. McCann, S. M., Haberland, P., Proc. Soc. Exp. Biol. and Med., 1959, v102, 319.

10. Sayers, M. A., Sayers, G., Woodbury, L. A., Endocrinology, 1948, v42, 379.

11. Dekanski, J., Brit. J. Pharmacol., 1952, v7, 562.

12. McCann, S. M., Endocrinology, 1957, v60, 664.

13. Royce, P. C., Sayers, G., Proc. Soc. Exp. Biol. and Med., 1960, v103, 447.

14. Schally, A. V., Lipscomb, H. S., Long, J. M., Dear, W. E., Guillemin, R., Endocrinology, 1961, v70, 478.

15. Guillemin, R., Schally, A. V., Texas Rep. on Biology and Med., 1963, v21, 541.

16. Rumsfeld, H. W., Porter, J. C., Arch. Biochem. Biophys., 1959, v82, 473.

Received October 18, 1965. P.S.E.B.M., 1966, v121.

Certain Aspects of Protein Metabolism in Hypercholesteremic Chickens* (30683)

SACHCHIDANANDA BANERJEE AND POTINENI NARASIMHA RAO
Research Division, Dey's Medical Stores Mfg. Private Ltd., Calcutta, India

Persistent hypercholesteremia is likely to produce changes in the metabolic pathways of proteins. It was, therefore, our interest to study the free amino acids of serum and aorta and the glutamic-oxalacetic-transaminase (GOT) and glutamic-pyruvic-transaminase (GPT) in the serum, aorta and liver of hypercholesteremic chickens. Liver was selected since it is an important site of trans-

* The investigation was carried out in the Physiology Department of the S.M.S. Medical College, Jaipur, India. The Council of Scientific & Industrial Research, New Delhi, financed the present investigation.

amination reactions. The study of GOT and GPT was preferred over other transaminases in order to understand the interrelations of the amino acids such as glutamic acid, aspartic acid and alanine with their respective ketoacids which form the connecting links between protein and carbohydrate metabolism. Further, these transaminases are more soluble and readily extractable(1).

Materials and methods. Twelve 2-months-old male White Leghorn chickens, of weights varying between 800-1200 g, were fed a basal diet mixed with sesame oil (10%) and cholesterol (1%) for 8 weeks so as to produce hypercholesteremia. The control group of birds of the same number, age, sex and weight received only the basal diet for the same period. The diet consisted of wheat flour 64 parts, ground Bengal gram (*Cicer arietinum*) 20 parts, commercial casein 12 parts, calcium carbonate 3 parts and common salt 1 part. The diet contained 2% fat, 19% protein, 65% carbohydrate and 1% fiber. Cholesterol of the diet was 15 mg per 100 g possibly derived from crude casein. In addition, the birds were fed 5 mg ascorbic acid daily and 2 drops of a concentrate of vit A twice a week.

After an overnight fast, blood was collected by cardiac puncture partly in heparinized tubes for estimation of plasma cholesterol and the rest was allowed to clot for the separation of serum which was used for estimation of serum proteins, free amino acids and transaminases. The birds were subsequently sacrificed by decapitation and a portion of aorta and liver were removed. Homogenates of aorta and liver were prepared with ice-cold distilled water. An aliquot of the homogenate was dried to constant weight to determine the dry weight of the tissue used. The homogenate was used for determination of GPT(2) and GOT(3). Enzyme activities are expressed in units/100 ml. One unit of activity is defined as producing a color intensity corresponding to 1 γ pyruvic acid/ml serum. The activities of liver and arterial transaminases are expressed in terms of γ pyruvic acid/mg dry wt of the tissue. The free amino acids of serum and aorta extracted (4) with the modification that the aqueous extract of the aorta was evaporated to dryness at 60°C and the residue dissolved in 10% isopropanol. The amino acids were separated by 2-dimensional paper chromatography and estimated(5). Total serum proteins were determined by the micro-Kjeldahl method. Total plasma cholesterol was also estimated(6).

Results. Sixteen amino acids were identified on the chromatograms obtained with serum samples. Tryptophan was not detected. Serum concentrations of glycine and proline were decreased. There was no change in the level of serum glutamic acid, and all the remaining amino acids increased when the birds developed hypercholesteremia. The free amino acids of the aorta decreased in hypercholesteremic chickens. Concentrations of lysine, serine, proline and glutamic acid remained unchanged. Aorta of both the normal and hypercholesteremic chickens did not contain tryptophan, hydroxyproline, methionine, isoleucine and histidine in the free form or contained them in too low a quantity to be detected in the chromatogram (Table I). Total serum proteins diminished in hypercholesteremic chickens.

In the hypercholesteremic chickens, the activities of serum GOT and GPT were increased, GOT of liver increased while GPT activity diminished, and the transaminases in the aorta did not change (Table II).

Discussion. Histochemical examination of aorta in the hypercholesteremic chickens revealed atheromatous plaques in the intima of the aorta. The increase of GOT and GPT of serum and GOT of liver in hypercholesteremic chickens indicated that the rate of transamination reaction was increased. This possibly resulted in the increased serum levels of aspartic or other amino acids. No change in glutamic acid level of serum was observed despite its active participation in transamination reaction. Glutamine is present in highest concentration in serum and in most tissues. Glutamine is formed from glutamate by the action of the enzyme glutamine synthetase. The absence of any change in the serum level of free glutamic acid in hypercholesteremia might be due to its conversion to glutamine. With the development of fatty liver in hyper-

TABLE I. Free Amino Acid Composition of Serum and Aorta of Normal and Hypercholesteremic-Atherosclerotic Chickens.

Amino acids	Serum (mg/100 ml) Normal (11)	Serum (mg/100 ml) Atherosclerotic (10)	t	Aorta (γ/g wet wt) Normal (10)	Aorta (γ/g wet wt) Atherosclerotic (9)	t
Lysine	1.58 ± .24	5.67 ± .31	10.5	3.50 ± .33	2.77 ± .22	1.83*
Histidine	3.33 ± .35	5.98 ± .36	5.2	—	—	
Arginine	3.39 ± .39	6.85 ± .38	6.3	10.30 ± .64	5.90 ± .26	6.42
Aspartic acid	1.94 ± .22	3.78 ± .10	7.7	13.23 ± .64	7.51 ± .35	7.87
Glutamic acid	5.45 ± .66	6.44 ± .56	1.2*	13.64 ± .73	11.96 ± .54	1.85*
Threonine	2.50 ± .16	5.86 ± .13	16.1	5.70 ± .28	3.46 ± .14	7.11
Serine	2.64 ± .28	3.69 ± .28	2.6	11.84 ± .54	10.82 ± .53	1.34*
Glycine	4.70 ± .48	3.30 ± .28	2.5	17.42 ± .64	8.25 ± .38	12.34
Tyrosine	1.78 ± .31	6.29 ± .44	8.7	4.89 ± .28	3.62 ± .19	4.31
Proline	6.77 ± .62	4.61 ± .48	4.0	14.35 ± .56	13.69 ± .63	.78*
Hydroxyproline	2.65 ± .23	4.15 ± .19	5.1	—	—	
Methionine	3.19 ± .44	5.79 ± .53	3.7	—	—	
Valine	5.32 ± .36	8.72 ± .52	5.2	11.98 ± .41	9.27 ± .24	5.78
Phenylalanine	1.09 ± .01	2.60 ± .27	4.3	2.21 ± .14	1.42 ± .11	4.41
Alanine	4.99 ± .35	7.77 ± .23	6.7	13.52 ± .46	10.63 ± .36	4.95
Leucine	3.07 ± .23	4.89 ± .43	3.7	4.75 ± .19	3.11 ± .16	6.57

Figures in parentheses = number of animals.
— indicates not detectable in the chromatogram.
* Except these values, other t values are significant at 5% level.
Values = Mean ± S.E.

TABLE II. Plasma Cholesterol, Total Serum Proteins, and GOT and GPT Activities in Serum, Liver and Aorta of Normal and Hypercholesteremic-Atherosclerotic Chickens.

	Normal (10)	Atherosclerotic (9)	t
Plasma cholesterol (mg/100 ml)	126 ± 6	1137 ± 52	19.36
Total serum proteins (%)	4.68 ± .04	4.17 ± .14	3.52
Serum GOT (units/100 ml)	61.2 ± 3.8	74.5 ± 4.5	2.28
Liver GOT (γ/mg dry wt)	11.6 ± 1.1	16.7 ± 1.4	2.84
Aorta GOT (γ/mg dry wt)	2.0 ± .14	2.26 ± .16	.73*
Serum GPT (units/100 ml)	4.5 ± .34	6.5 ± .73	8.05
Liver GPT (γ/mg dry wt)	2.2 ± .10	.62 ± .14	4.5
Aorta GPT (γ/mg dry wt)	.10	.04	

Figures in parenthesis indicate number of samples.
Values = Mean ± S.E.
* Except this value, other values of t were significant at 5% level.

cholesteremia(7), the capacity of the liver oxidatively to deaminate amino acids is reduced, which might be responsible for the increased concentrations of amino acids in the serum. The reduction of serum glycine might be due to its conversion into serine which was found to be increased. The decrease in serum proline might be due to its incorporation into the newly formed collagen of the atherosclerotic aorta.

The decrease in concentration of amino acids in the aorta of hypercholesteremic chickens which also showed atheromatous plaques, was possibly due to increased synthesis of collagen(8). As tryptophan is bound to a non-dialysable plasma protein(9) free tryptophan could not be detected. The alterations in the free amino acid composition indicated that chemical changes accompany the morphological alterations during the development of atherosclerosis as a result of hypercholesteremia. The decline in serum proteins might be due to defective synthesis by the liver whose function is likely to be impaired due to accumulation of fat.

Summary. Glutamic-oxaloacetic-transaminase (GOT) and glutamic-pyruvic-transaminase (GPT) of serum, aorta and liver and free amino acid composition of the serum and aorta were determined in normal and hypercholesteremic-atherosclerotic chickens. GOT and GPT of serum and GOT of liver increased with the onset of atherosclerosis suggesting an overall increase in the rate of

transamination reaction in the body. The activities of the arterial transaminases did not change appreciably. Concentrations of most of the free amino acids increased in the serum and diminished in the aorta of hypercholesteremic-atherosclerotic chickens. Chemical changes accompany the morphological alterations during the progress of atherosclerosis.

1. Green, D. E., Leloir, L. F., Nocito, V., J. Biol. Chem., v161, 559.

2. Wroblewski, F., Caband, P., Am. J. Clin. Path., 1957, v27, 223.

3. Caband, P., Leeper, R., Wroblewski, F., ibid., 1956, v26, 1101.

4. Awapara, J., Arch. Biochem., 1948, v19, 172.

5. Bhown, A. S., Banerjee, S., Indian J. Exp. Biol., 1963, v1, 164.

6. Abell, L. L., Levy, B. B., Brodie, B. B., Kendall, F. E., J. Biol. Chem., 1952, v195, 357.

7. Bandyopadhyay, A., Banerjee, S., Indian J. Exp. Biol., 1964, v2, 16.

8. Engel, A. F., Pollak, O. J., Experientia, 1961, v17, 210.

9. Holmberg, C. G., Blomstrand, R., Ann. Rev. Biochem., 1959, v28, 321.

Received September 20, 1965. P.S.E.B.M., 1966, v121.

Effects of Biotin Deficiency on Pyruvate Metabolism.* (30684)

S. R. WAGLE

Department of Pharmacology, Indiana University School of Medicine, Indianapolis

The studies of Lynen *et al*(1) and Wakil *et al*(2) have demonstrated that biotin serves as a co-enzyme for several enzymatic carboxylation reactions. These specifically include the formation of methylmalonyl CoA from propionyl CoA and CO_2(3), malonyl CoA from acetyl CoA and CO_2(4) and conversion of methylcrotonyl CoA and CO_2 to β-methyl-glutaconyl CoA(5). Terroine(6) has reported that in acute biotin deficiency there is an increase in blood pyruvic acid levels. Such changes may result from decreased pyruvate utilization. In the metabolism of pyruvate to lactate or acetyl CoA biotin is presumably not involved; however, Keech and Utter(7,8) have recently described the properties of a pyruvic carboxylase which converts pyruvate and CO_2 into oxaloacetate in chicken liver preparations. Possible involvement of biotin in glucose metabolism has been suggested(9), and recently we have reported(10) reduced incorporation of $^{14}CO_2$ into glucose in biotin-deficient rats. The conversion of pyruvate to phosphoenolpyruvate (PEP) is a crucial step in the synthesis of glycogen or glucose from precursors of pyruvate in liver. The formation of phosphoenolpyruvate could occur through the reversal of the glycolytic reaction catalyzed by pyruvate kinase, but the physiological significance has been questioned on energetic grounds(11). The fact that phosphoenolpyruvate could be formed from oxaloacetate by phosphoenolpyruvate carboxykinase(12) would suggest that PEP might be formed by a route which bypasses pyruvate kinase. In addition oxaloacetate can be formed by carboxylation of pyruvate by pyruvate carboxylase(7,8). These reactions are as follows:

$$\text{I Pyruvate} + CO_2 + \text{ATP} \xleftarrow[\text{Pyruvate carboxylase}]{\text{Acetyl CoA, } Mg^{++}} \text{oxaloacetate} + \text{ADP} + P_i$$

$$\text{II Oxaloacetate} + \text{ITP (or GTP)} \xleftarrow[\text{PEP carboxykinase}]{Mn^{++}} \text{Phosphoenol pyruvate} + CO_2 + \text{IDP (or GDP)} + P_i$$

It is reported(7,8) that pyruvic carboxylase is a biotin-containing enzyme, and that this reaction may become rate limiting in glucose formation from lactate or pyruvate in biotin-deficient animals. To test this hypothesis liver slices from normal and biotin-deficient rats were incubated with pyruvate-2-^{14}C and

* This investigation was supported by USPHS Research Grants AM-04790, AM-08242, and CA-06276 from Inst. of Arthritis and Metab. Dis. and Nat. Cancer Inst.