wall," from which it is slowly released and blocks the receptors through which the effect of norepinephrine is thought to be mediated. Indeed it has been found that catecholamines can be stored in tissues innervated by sympathetic nerve fibers and that these tissues can take up a considerably greater amount than their usual catecholamine content(5). Furthermore, it has been reported that the loss of norepinephrine from tissues which occurs after sympathetic denervation results in supersensitivity to the amine(6).

A similar increase in sensitivity to norepinephrine occurs after guanethidine(7) and this might possibly explain the delay in norepinephrine tachyphylaxis observed in the present study. The mechanism of this type of hypersensitivity, however, does not seem to be explained by the properties of guanethidine, which are concerned with depletion of tissue catecholamines and interruption of sympathetic transmission(8,9). Indeed, while both guanethidine and reserpine deplete tissues of catecholamines the acute sensitization to norepinephrine is observed only after guanethidine(8,10). Furthermore, the pressor response to norepinephrine is increased after guanethidine in reserpinized dogs and in dogs with spinal cord section(11). These facts would seem to favor the hypothesis that a direct action of guanethidine on the receptors might account for the potentiating effect of the catecholamine.

On the other hand, Hertting *et al*(12) have demonstrated in acute experiments that in guanethidinized animals administration of H^3-labeled norepinephrine results in an elevation of norepinephrine plasma levels and a decrease in tissue concentration of norepinephrine. Therefore, by exposing the receptors to a higher plasma level of norepinephrine guanethidine might effect a greater pressor response of norepinephrine.

Summary. Pressor responses to repeated injections of norepinephrine were recorded in a control group of dogs and in dogs pretreated with guanethidine. The results indicate that guanethidine maintains the pressor response of norepinephrine, *i.e.*, delays tachyphylaxis to norepinephrine.

1. Rosenthale, M. E., DiPalma, J. R., J. Pharm. Exp. Therap., 1962, v136, 336.

2. Perez-Reyes, M., Lipton, M. A., Proc. Soc. Exp. Biol. and Med., 1963, v112, 181.

3. Muelheims, G. H., Zekert, H., Walter, K. E., Arch. Int. Pharmacod. et Therap., 1964, v149, 62.

4. Burn, J. H., Rand, M. J., Brit. Med. J., 1959, v1, 394.

5. Stromblad, B. C. R., Nickerson, M., J. Pharm. Exp. Therap., 1961, v134, 154.

6. Cooper, T., William, V. L., Potter, L. T., Hanlon, C. R., Physiologist, 1963, v6, 160.

7. Maxwell, R. A., Plummer, A. J., Schneider, F., Povalski, H., Daniel, A. I., J. Pharm. Exp. Therap., 1960, v128, 22.

8. Krayer, O., Alper, M. H., Paasonen, M. K., ibid., 1962, v135, 164.

9. Gaffney, T. E., Chidsey, C. A., Braunwald, E., Circ. Res., 1963, v12, 264.

10. Trendelenburg, U., Weiner, N., J. Pharm. Exp. Therap., 1962, v136, 152.

11. McCubbin, J. W., Kaneko, Y., Page, I. H., ibid., 1961, v131, 346.

12. Hertting, G., Axelrod, J., Whitby, L. G., ibid., 1961, v134, 146.

Received May 14, 1965. P.S.E.B.M., 1965, v120.

Selenium and Rabbit Skeletal Muscle Aldolase and Myosin.* (30452)

KENNETH P. MCCONNELL AND D. M. ROTH

Radioisotope Service, Veterans Administration Hospital and Biochemistry Department, School of Medicine, University of Louisville, Louisville, Ky.

There is a relationship between nutritional selenium deficiency and muscular dystrophy, since a selenium deficient ration will produce muscle dystrophy in lambs(1,2) and cattle (3,4). Muth(4), as well as others(5,6), has shown that sodium selenate when fed to sup-

* This investigation was supported in part by grant A-4445 from Nat. Inst. Health.

TABLE I. Incorporation of Selenium-75 into Rabbit Muscle Myosin.

No. of "crystallizations"	μmoles Pi* per hr (per mg protein) (A)	cpm (per mg protein) (B)	Ratio Se-75 act. (B) / Enzyme act. (A)	% of total muscle Se-75 activity in myosin†	Protein-bound selenium, %
1	90	34	.38	29.6	92.6
2	139	41	.29	35.4	92.4
3	142	39	.27	33.6	94.5

* Inorganic phosphate.

† Assuming that each gram of fresh muscle contains 80 mg myosin(22) and using the values for cpm per mg myosin and cpm per g fresh muscle (9,227 ± 752.7), % of total muscle Se-75 activity in myosin (column 5) was estimated.

ply 0.1 ppm selenium in the ration, exerted a strong protective effect against white muscle disease in domestic animals(7).

It has been reported that the levels of ATP in muscle of animals with nutritional (vitamin E deficiency) dystrophy, when compared with normals, are significantly decreased in the rabbit(8,9), mouse(10) and humans(11). The decrease in ATP concentration may be caused by an increase in ATPase activity in the muscle of dystrophic animals(8,12). Concurrently, creatine concentration in muscle decreases with the progress of the disease(8). The inability of dystrophic muscle to phosphorylate creatine appears most likely to be the result of a deficiency in ATP. Srivastava and Berliquet(13) report a decrease in the specific activity of aldolase in hereditary muscular dystrophic mice. However, they found an increase in aldolase at the later stages of the disease when expressed on a per mg nitrogen basis.

Since it has been estimated that about a third of the total body selenium is in total body musculature and there is an apparent relationship between muscle biochemistry and selenium, it was of interest to investigate the incorporation of selenium into ATPase (myosin) and aldolase of rabbit skeletal muscle and determine the effects of selenium on the activity of these enzymes. It was found that Selenium-75 was incorporated into aldolase and ATPase and that added selenium ($SeO_3^=$) had no significant effect on activity of these enzymes.

Methods. Young adult rabbits (2 to 3 kg) were injected subcutaneously with $H_2Se^{75}O_3$ which contained 0.3 mc (10 μg Se) in myosin experiments and 0.29 mc (17 μg Se) in aldolase experiments. Twenty-four hours later the animals were sacrificed and myosin was extracted from the skeletal muscle by the KCl phosphate buffer method described by Kessler and Spicer(14). Aldolase was prepared by the method of Taylor *et al*(15). ATPase activity of myosin was determined by the method of Gergely(16) and aldolase activity by the method of Warburg and Christian(17) used by Taylor(15a). A unit of enzyme activity was defined for aldolase as the transformation of one mole of fructose diphosphate per minute; for ATPase activity as the liberation of 1/22.41 μmoles of inorganic phosphate per hour at 39°. Radioactive assay of Selenium-75 has been described(18) and selenium was determined by neutron activation analysis according to the method of Leddicotte and Reynolds(19). Protein concentration in solutions of aldolase and myosin was determined by the method of Lowry *et al* (20). Protein-bound selenium was determined by the trichloroacetic acid method(21). The efficiency for counting Selenium-75 was 43%.

Results. The incorporation of Se-75 into rabbit muscle myosin is shown in Table I where the results are expressed as cpm per mg protein. It will be noted that the second and third crystallizations resulted in a constant ratio of selenium to enzyme activity (column 4) which is interpreted as evidence that selenium was incorporated into the myosin molecules. About 33% (Table I, column 5) of the Se-75 found in muscle was present under experimental conditions described here in the contractile muscle protein myosin. Table II shows the selenium and activity of rabbit muscle aldolase after each of 5 consecutive crystallizations. There was no

TABLE II. Selenium-75 and Enzyme Activity of Rabbit Muscle Aldolase.

No. of "crystallizations"	Aldolase activity (units* $\times 10^{-6}$/mg protein)	Se-75 radioactivity (cpm/mg protein)	Se-75 radioactivity/aldolase activity (cpm/units* $\times 10^{-6}$)
1	2.99	360	120.0
2	4.33	223	51.5
3	4.20	178	42.4
4	4.88	166	34.0
5	3.24	132	40.7

* 1 Unit = 1 mole fructose diphosphate transformed/min.
$= (\Delta\ 1.00\ \text{O.D.}_{340\ m\mu}/\text{min})/6.22 \times 10^6$.

enzymatic activity in control experiments where DPN and D-glyceraldehyde-3 phosphate dehydrogenase were not added.

Dialysis of the purified preparations of myosin against buffer solutions at various pH's indicated that the incorporated Se-75 was not dialyzable in acid or neutral pH's. Furthermore, incorporated Se-75 was less stable in the higher or alkaline ranges for at pH 8.6 and 9.9 approximately 10 and 23%, respectively, were dialyzable. Aldolase had similar dialysis properties. Protein-bound selenium in myosin, determined by the trichloroacetic acid method, was 92 to 94%; aldolase 95 to 96%. These findings were similar to those obtained on selenized plasma, liver and leucocyte proteins(18,21,23,24).

From past experience with a variety of proteins it appears likely that selenium was present in trace amounts in muscle myosin and aldolase. Myosin and aldolase crystallized from normal healthy rabbits with no known exposure to selenium, as far as we were able to ascertain, was dialyzed for 24 hours against distilled water and then lyophilized. The enzymes were analyzed for selenium(19). Results obtained from 6 determinations revealed that myosin contained about 22.7 $\pm$ 6.7 μg selenium per gram of myosin protein. Other trace elements assayed in myosin were lanthanum (26 μg per gram), and manganese (2.53 μg per gram). Aldolase contained 12.6 $\pm$ 2.8 μg selenium per gram.

Since selenium was shown to be incorporated into myosin and aldolase when administered as Se-75, and since trace amounts of stable selenium were present in myosin and aldolase, it was of interest to test *in vitro* the ATPase activity of myosin and aldolase activity from non-selenized animals in the presence of graded amounts of non-radioactive selenium as selenite. Concentrations of selenium ($SeO_3^=$) from 10^{-2} to 10^{-6} M had no significant inhibitory or activation effects on either the ATPase activity of myosin (control 52 to 59 units; added $SeO_3^=$ 50 to 62 units) or aldolase (control 16.1 and 17.4 units; added $SeO_3^=$ with reduced glutathione 16.7 to 17.7 units; control 16.1 units without reduced glutathione 11.9 to 14.8 units) obtained from normal nontreated rabbits.

Using the value of 12.6 μg selenium per gram aldolase and 22.7 μg selenium per gram myosin, and 149,000 and 440,000 M.W. for aldolase and myosin, respectively, it was estimated that there was less than one atom selenium per mole enzyme. Despite the fact that selenium has been shown to be incorporated in these enzymes, the value of less than one atom selenium per mole enzyme appears to be stoichiometrically insignificant. Experiments described here on the negative effects of added selenium on aldolase and ATPase activity support this contention.

Summary. Injection of rabbits with sublethal, sub-toxic amounts of Selenium-75 resulted in the incorporation of selenium into the muscle protein myosin and aldolase. About a third of the muscle Selenium-75 under conditions of our experiments was present in the protein myosin. Myosin isolated from the muscle of animals not treated with selenium contained 22.7 μg selenium per gram myosin and aldolase from animals not treated with selenium contained 12.6 μg selenium per gram. This quantity plus the amount incorporated in the experiments amounts to considerably less than 1 mole Se per mole myosin or per mole aldolase. This, coupled with the observation that selenium between 10^{-2} M and 10^{-6} M has no effect on catalytic activity of these enzymes, suggests that this trace element does not play a role in the action of these enzymes.

The author wishes to express his appreciation to Dr. Calvin Lang for his helpful assistance.

1. Muth O. H., Oldfield, J. E., Remmert, L. F., Schubert, J. R., Science, 1958, v128, 1090.

2. Proctor, J. F., Hogue, D. E., Warner, R. G., J. Animal Sci., 1958, v17, 1183.

3. Muth, O. H., Oldfield, J. E., Schubert, J. R., Remmert, L. F., Am. J. Vet. Res., 1959, v20, 231.

4. Muth, O. H., Schubert, J. R., Oldfield, J. E., ibid., 1961, v22, 466.

5. Kuttler, K. L., Marble, D. W., ibid., 1960, v21, 437.

6. Lagace, A., J.A.V.M.A., 1961, v139, 188-190.

7. Muth, O. H., ibid., 1963, v142, 272.

8. Sarks, Nirmal K., Srivastava, U., J. Nutrition, 1964, v83, 193.

9. Grigoreva, V. A., Medovar, E. N., Ukrain. Biokhim. Zhur., 1959, v31, 351.

10. Zymaris, C. M., Saifer, A., Volk, B. W., Nature, 1960, v188, 323.

11. Bonetti, E., Taschi, F. N., Levi, M., Sperimentale, 1954, v104, 315.

12. Pennington, R. J., Biochem. J., 1961, v80, 649.

13. Srivastava, U., Berliquet, L., Can. J. Biochem., 1964, v42, 1301.

14. Kessler, V., Spicer, S. S., Biochem. et Biophys. Acta, 1952, v8, 474.

15. Taylor, J. F., Biochemical Preparations, 1957, v5, 12.

15a. ———, Methods in Enzymology, Academic Press, Inc., New York, 1955, v1, 312.

16. Gergely, J., J. Biol. Chem., 1953, v200, 543.

17. Warburg, O., Christian, W., Biochem. Z., 1943, v314, 149.

18. McConnell, K. P., Wabnitz, C. H., J. Biol. Chem., 1957, v226, 765.

19. Leddicotte, G. W., Reynolds, S. A., Nucleonics, 1951, v8, 2.

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

21. McConnell, K. P., Van Loon, E. J., ibid., 1955, v212, 747.

22. Haurowitz, F., Chemistry and Biology of Proteins, Academic Press, 1950, p161.

23. McConnell, K. P., Cooper, B. J., J. Biol. Chem., 1950, v183, 459.

24. McConnell, K. P., Texas Rep. on Biol. & Med., 1959, v17, 120.

Received April 26, 1965. P.S.E.B.M., 1965, v120.

The Disposition of Orally Administered Cholestyramine-C^{14}. (30453)

D. G. GALLO AND A. L. SHEFFNER

Department of Nutritional Biochemistry, Mead Johnson Research Center, Evansville, Ind.

Cholestyramine, a strongly basic anion exchange resin, has been shown to be an effective hypocholesteremic agent(1,2) and to be useful in the relief of pruritus associated with biliary cirrhosis(3,4,5). It has been generally assumed that ion exchange resins are not absorbed from the gastrointestinal tract due to their high molecular weight and extreme insolubility. This assumption was supported by the absence of toxic reactions associated with long-term administration of cholestyramine. In the present investigation the question of whether cholestyramine is absorbed from the gastrointestinal tract was studied directly by oral administration of C^{14}-labeled cholestyramine to rats, and subsequent measurement of C^{14} in the blood, tissues, excretions and expired air of the treated animals. The results indicate that cholestyramine resin is not absorbed from the gastrointestinal tract of rats.

Materials and methods. Cholestyramine-C^{14}. The carbon skeleton of cholestyramine is composed of a copolymer of styrene and divinylbenzene; the strongly basic character of the resin is due to the presence of aromatic quarternary ammonium groups. The cholestyramine-C^{14} used in these studies was synthesized by Dow Chemical Co., Midland, Mich., using styrene-8-C^{14} as one of the starting materials. The resin product was ground and washed with dilute HCl and hot distilled water to remove radioactive impurities. It was then lyophilized and ground further to a size small enough to pass through a 200-mesh seive. The specific activity of the labeled resin was approximately 0.14 μc per mg.

Experimental design. The experiments were designed to study the possible absorption of orally administered cholestyramine-C^{14} under two types of experimental conditions: (1)