of slices, to introduce iodoalbumin into the extravascular exchangeable pool of various organs, significant breakdown did not occur. Breakdown of exogenous S^{35} biosynthetically labeled homologous albumin and globulin occurred only in slices of small intestine.

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Incorporation of P^{32} into the O-Phosphodiester of L-Serine and Ethanolamine in the Turtle.* (25679)

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Roberts and Lowe(1) reported presence of O-phosphodiester of L-serine and ethanolamine ([2-aminoethyl]-[L-2-amino-2-carboxyethyl]-phosphoric acid) in turtle tissue. This compound (SEP) also has been found in tissues of snake and alligator. SEP and the DL-serine phosphodiester have been synthesized (2). A similar compound and a phosphagen thereof have been found in the earthworm in which the 2-aminoethyl group is replaced by 2-guanidoethyl or phosphoguanidoethyl group (3). The purpose of our study was to obtain information on biosynthesis of SEP in turtle tissue. A preliminary report of this work has been made(4).

Results. Comparison of isolated and synthetic SEP. Mobilities of isolated SEP(1) and the synthetic compound(2)† were compared in 4 solvent systems by descending chromatography: (a) phenol saturated with water; (b) methyl Cellosolve-water (9:1); (c) acetone-acetic acid-water (4:2:2); and (d) n-butanol-acetic acid-water (8:20:20). The compounds were visualized by spraying with ninhydrin. The results are shown in Fig. 1. In each solvent the compound on the left was the isolated material, that on the right the synthetic material, and in the middle a mixture of the two. Approximately 200 γ of each were used. In each case the natural and synthetic materials showed identical mobilities.

Incorporation of P^{32} orthophosphate into SEP. After drilling holes through plastron above the heart with a trephine, approximately 1 mc of P^{32} orthophosphate in isotonic saline was injected into the ventricle of each of 5 unanesthetized turtles (*Pseudemys elegans*), which were sacrificed at 9, 26, 48, 76 and 99 hours, respectively, after injections. The holes were covered with adhesive tape after injection to prevent contamination and leakage of body fluids. The various organs were removed and homogenized in 10 volumes of 80% ethyl alcohol. After centrifugation the clear supernatant fluids were evaporated to dryness under stream of nitrogen. Residues were taken up in 50% alcohol so that 0.1 ml of the mixture was equivalent to 100 mg of original fresh weight of tissue, and 0.1 ml aliquots were spotted on paper for chromatography. Two-dimensional chromatograms were run routinely in phenol-water and

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† Kindly supplied by Prof. David Lipkin.

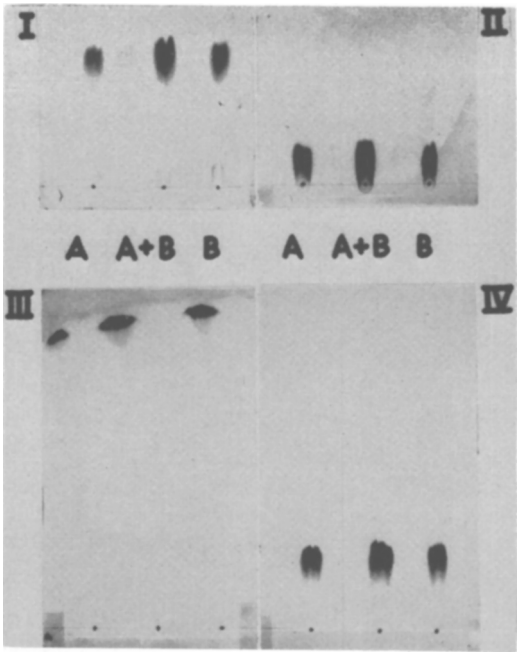

FIG. 1. Mobilities of isolated (A) and synthetic (B) SEP in phenol saturated with water, I; methyl Cellosolve - water (9:1), II; acetone - acetic acid - water (4:2:2), III; and n-butanol - acetic acid - water (8:20:20), IV.

lutidine-water or methyl Cellosolve-water systems. Although use of methyl Cellosolve for the second dimension is in some regards inferior to lutidine, it permits separation of SEP from ethanolaminephosphate (EP), which the lutidine system does not accomplish. After chromatography the sheets were placed in contact with Type K industrial X-ray film for 7 days and were then sprayed with 0.2% ninhydrin in butanol. Photographs were made of both chromatograms and radioautographs. Tissues studied in this manner included liver, kidney, heart, brain, pancreas, sacculus rotundus (a glandular tissue located near the pancreas), and white and red muscle.

The results of ninhydrin sprayed chromatograms in Fig. 2, 4, and 6 illustrate previously observed finding(5) that patterns of free or easily extractable amino acids in extracts of tissues of a particular species differ from each other. The same tissues from different turtles showed somewhat greater variability than is found in mammalian or avian species. The highest concentrations of SEP were found in extracts of red (Fig. 2) and white muscle and heart (Fig. 4), which tissues also contained highest levels of taurine. Smaller amounts of SEP were found in other tissues. In all tissues only small quantities of EP were detected.

Incorporation of P^{32} took place into a number of constituents detectable on radioautographs. However, attention will be drawn

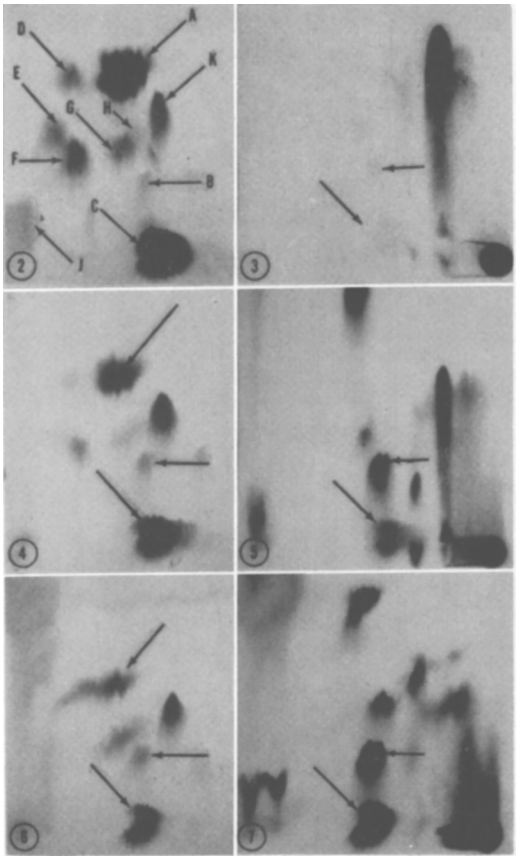

FIG. 2, 4, and 6. Paper chromatograms of extracts obtained from 100 mg of original fresh wt of red muscle, heart, and kidney, respectively, from a turtle 99 hr after administration of approximately 1 mc of P^{32}.

FIG. 3, 5, and 7. Corresponding radioautographs. The solvent directions on chromatograms are phenol - water from right to left and methyl Cellosolve - water from bottom to top.

Constituents identified on Fig. 2 are: Taurine, A; ethanolamine-phosphate (EP), B; phosphodiester of serine and ethanolamine (SEP), C; alanine, D; glycerylphosphorylethanolamine, E; glutamine, F; glycine, G; serine, H; arginine, J; glutamic acid, K. On all subsequent chromatograms arrows point to taurine, EP, and SEP and on the radioautographs to EP and SEP, the direction and position of arrows clearly identifying these constituents.

only to SEP and EP. Kidney (Fig. 6 and 7) and heart (Fig. 4 and 5), which showed highest degree of labeling of SEP, also had the greatest radioactivity in EP. Liver, heart (Fig. 4 and 5), and kidney (Fig. 6 and 7), showed greatest number of P^{32}-labeled constituents on the radioautographs. In all instances visible labeling of EP occurred before that of SEP. Under conditions employed kidney, liver, heart, and brain showed detectable P^{32} labeling in EP at 9th hour after injection of P^{32} orthophosphate, while autographs showed detectable radioactivity in SEP only at 26, 48, 76, and 76 hours, respectively, in the same extracts. The pancreas, sacculus rotundus, red muscle, and white muscle showed detectable label in EP at 26, 48, 48, and 48 hours, respectively, while only in the 99 hour sample was there any radioautographic evidence of labeling of SEP in these tissues. While the quantity of EP detectable on chromatograms was less than that of SEP in all tissues, the extent of labeling of EP was approximately the same as that of SEP in kidney and heart and greater in other tissues, indicating the much higher specific activity of EP. The results did not lead to identification of any P^{32} containing compounds other than EP which might be implicated as a possible precursor of SEP. It is interesting that red (Fig. 2 and 3) and white muscle, which contained the largest quantities of extractable SEP, showed least radioactivity in SEP and EP and the smallest number of labeled constituents on radioautographs.

Streak preparations of pooled extracts from tissues of the 99 hour turtle were made on 10 large sheets of paper, extract equivalent to 1 g being placed on each sheet, and were run one-dimensionally in methyl Cellosolve. Marker strips on the edge were sprayed with ninhydrin and the SEP band was outlined and eluted with water, evaporated to dryness under a fan and lamp. The dried eluate was taken up in 0.9 ml of water and 0.3 ml were employed for preparation of the DNP derivative. Chromatography in dioxane-ammonia solvent (4:1) separated the DNP derivatives of synthetic serine phosphate, ethanolamine phosphate, and SEP. It was found upon radioautography of the chromatograms of the isolated material that radioactive areas corresponded exactly with the DNP derivative of SEP on the chromatograms, thus showing unequivocally that the P^{32} label was located in SEP.

In vitro experiments. *In vitro* experiments with intact beating hearts, kidney and liver slices, brain homogenates, etc., incubated under a variety of conditions did not give unequivocal evidence of incorporation of P^{32} into SEP or of C^{14} from labeled glycine, acetate, or glucose. Only in the incubation of the erythrocytes of turtle blood with P^{32} in plasma was evidence obtained for a small *in vitro* incorporation of the label into SEP after 8 and 12 hours of incubation at 37°. Thus, although present results show that SEP can be synthesized in the turtle, no mechanism for biosynthesis of SEP can be postulated as yet.

Summary. 1. It was shown that intracardially administered P^{32} orthophosphate is incorporated into O-phosphodiester of L-serine and ethanolamine (SEP) and ethanolaminephosphate (EP) in tissues of the turtle. 2. Kidney and heart showed highest degree of labeling of SEP and EP. White and red muscle exhibited smallest uptake of P^{32} into SEP, although containing larger amounts of this compound than other tissues examined. 3. Only incubation of intact erythrocytes in plasma for prolonged periods, of a variety of *in vitro* systems tested, gave any evidence of P^{32} incorporation into SEP.

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