strains (Table II).

Summary. Twenty-three strains of fibroblasts derived from normal rabbit and chick embryo tissue were serially propagated on glass in a variety of media. All except 3 strains failed to proliferate for more than 8 to 12 subcultures. The 3 successful strains were derived from adult testis, embryonic skin-muscle and adult muscle respectively and continue to proliferate at a constant rate after 38 to 61 subcultures. Vaccinia virus multiplies in all 3 strains of fibroblasts resulting in the complete destruction of cells.

1. Robbins, F. C., and Enders, J. F., *Am. J. Med. Sci.*, 1950, v220, 316.

2. Sanders, M., *Arch. Path.*, 1953, v56, 148.

3. Swim, H. E., and Parker, R. F., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 549.

4. ———, *ibid.*, 1953, v83, 577.

5. Fischer, A., Astrup, T., Ehrensvard, G., and Oehlenschlager, V., *ibid.*, 1948, v67, 40.

6. Healy, G. M., Fisher, D. C., and Parker, R. C., *Can. J. Biochem. Physiol.*, 1954, v32, 327.

7. Earle, W. R., and Highhouse, F., *J. Nat. Cancer Inst.*, 1954, v14, 841.

8. Rivers, T. M., and Ward, S. M., *J. Exp. Med.*, 1933, v58, 635.

9. Reed, L. J., and Meunch, H., *Am. J. Hyg.*, 1938, v27, 493.

10. Eagle, H., *Science*, 1955, v122, 501.

11. Morgan, J. F., Morton, H. J., and Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 1.

12. Burnet, F. M., *J. Exp. Med.*, 1936, v63, 685.

13. Perry, V. P., Evans, V. J., Earle, W. R., Hyatt, G. W., and Bedell, W. C., *Am. J. Hyg.*, 1956, v63, 52.

14. Sanford, K. K., Likely, G. D., and Earle, W. R., *J. Nat. Cancer Inst.*, 1954, v15, 215.

Received June 27, 1956. P.S.E.B.M., 1956, v93.

Studies on Utilization of Sulfate Sulfur for Growth of the Chicken.*† (22708)

L. J. MACHLIN‡ AND P. B. PEARSON

Animal and Poultry Husbandry Research Branch, U. S. Department of Agriculture, Beltsville, Md.

It has been reported(1) that addition of sodium sulfate to a purified diet devoid of inorganic sulfur improved growth and feed efficiency of young chickens. Studies with S^{35}-sulfate have demonstrated that small amounts of sulfate sulfur are incorporated into cystine in the growing chicken(1,2) and laying hen (3), and larger portions into taurine by the chick embryo(4). In addition, the feathers of birds fed $Na_2S^{35}O_4$ contain a radioactive organic fraction which is not a sulfur amino acid and which has not been identified(1).

The purpose of these studies was to verify the growth response from sulfate sulfur, to determine whether such a response results from the conversion of sulfate sulfur to sulfur amino acids and to identify the radioactive compound appearing in the feathers after the feeding of $Na_2S^{35}O_4$.

Growth. The basal diet was a modification§ of that used by Gordon and Sizer(1), which contains 15% casein and 10% gelatin as the protein‖ source. Female New Hampshire X Silver Cornish crossbred chicks were fed the basal diet for 1 week. They were then weighed, divided into groups of 13 chicks of equal weight distribution and fed the experimental diets for 3 weeks. The chicks were reared under electrically heated brooders in wire-floored cages, in an air-conditioned room. Addition of 0.5% Na_2SO_4 to the basal

* This project was supported in part by the U. S. Atomic Energy Commission.

† An abstract of part of this work was published in *Poultry Sci.*, 1955, v34, 1209.

‡ Present address: Monsanto Chemical Co., St. Louis, Mo.

§ Wesson oil or lard was used as the fat. $MgCO_3$, $MnCl_2$ and copper acetate were used instead of the corresponding sulfates.

‖ The protein furnished about 0.54% methionine and 0.06% cystine. Since the estimate corresponds closely to the 0.132% total sulfur found by analysis the diet probably contained no inorganic sulfur.

TABLE I. Growth Response from Addition of 0.5% Na_2SO_4 to Low-Sulfur Diet.

Exp. No.	Basal diet	0.5% Na_2SO_4	Increase, %	Level of significance*
	Body wt (g) at 4 wk			
1	160	232	45	.01
2	157	174	11	none
3	192	237	23	.01
	175	210	20	.05
4	285	300	5	none
5	204	283	39	.01
	224	276	23	.01
6	228	274	20	.05
7	239	294	23	.05
8	230	262	14	none
9	245	284	16	.05
Avg	213	257	22	

* Probability using Students' "t" test.

diet resulted in a growth response in every trial which was usually statistically significant (Table I). Feathering of birds fed the basal diet appeared normal.

Utilization of $Na_2S^{35}O_4$. A group of chicks was fed the basal diet plus 0.4% DL methionine. At 2 weeks of age the chicks were fed this diet plus 5 mg of $Na_2S^{35}O_4$ (1.6 mc) per kilo. One and 2 weeks after the addition of radioactive sulfate, 5 chicks were killed and the S^{35} in the sulfate, cystine, methionine, and taurine fractions determined for individual carcasses. The methods were essentially those previously described(5), except that paper chromatography was employed for further purification of taurine following chromatography on Dowex-50 resin. A summary of these results is given in Table II. Only trace amounts of radioactive cystine or methionine could be detected at 1 week following feeding of radioactive sulfate. After 2 weeks there was a measurable amount of radioactivity in these amino acids. Since only 1.5% of the S^{35} ingested was recovered in the cystine and methionine it would not appear that the rate of incorporation of sulfur into these amino acids was sufficient to significantly reduce the requirement for these amino acids in the diet. Taurine-S^{35} accounted for about 19% of the S^{35} recovered in the birds after 1 week of sulfate-S^{35} feeding and 24% after 2 weeks. This increase would suggest that taurine has a longer biological half-life than sulfate. A portion of the organic-S^{35} could not be accounted for as either cystine, methionine or taurine. It is likely that some of this S^{35} was present as unidentified compounds. These probably arise from the metabolism of taurine (6). Taurine accounted for an average of 6.7% and sulfate 5.6% of the total sulfur of the carcass of 4-week-old chickens.

TABLE II. Recovery of S^{35} from Compounds Isolated from Carcass of Chickens Fed $Na_2S^{35}O_4$.*

Compound	Duration of feeding period (wk)† 1	2
	% of total S^{35} recovered	
Sulfate	74.5	57.8
Cystine	trace	1.4
Methionine	"	.1
Taurine	18.9	24.1
Remainder	6.6	16.6
Total	100.0	100.0
% of dose‡ administered	22.7	19.7

* 1.6 mc/g of diet.
† Chickens were 2 wk of age at start of radioactive feeding period.
‡ Based on avg feed consumed.

Feather compound. The feathers from the birds fed sulfate-S^{35} were analyzed after acid hydrolysis. Sulfate, cystine and methionine accounted for only 47% of the S^{35} in these feathers. The remainder of the S^{35} behaved similarly to taurine on Dowex-50 columns. After paper chromatography, however, it did not give the same R_f as taurine with all solvent systems. A brown pigment was present in fractions containing the unknown S^{35} compound. In order to avoid any complication from this pigment in the colored feathers of the chicks used, a second study was conducted with White Leghorn chicks. In this study it was found that almost all of the unknown S^{35} could be extracted from the ground feathers with hot water. This water extract was subjected to chromatography on Dowex-50(4), followed by paper chromatography with 5 different solvent systems. Essentially all of the non-sulfate-S^{35} in the extract was thereby identified as taurine-S^{35}. Approximately 54 mg% of taurine (on dry matter basis) was found(4) in these feathers after acid hydrolysis. This accounted for about 10% of the free amino nitrogen and 0.6% of the total sulfur in the feathers.

A third study was conducted with New Hampshire X Silver Cornish birds. The S^{35} compound was freed of contamination with pigment by eluting it from large sheets of Whatman 3MM filter paper after development of radioactive fractions with n-propanol-1N NH_4OH (5:1). When this unknown S^{35} was rechromatogramed 90% of the S^{35} was found at the R_f values corresponding to taurine and the R_f was not changed after treatment with H_2O_2. With some chromatograms a small second peak of S^{35} was detected suggesting the presence of another sulfur compound. However, most of the unknown S^{35} was apparently taurine whose detection was obscured by the presence of feather pigment.

The significance of taurine in feathers is not known. It has been suggested(6) that end-products of metabolism such as uric acid and creatinine are "trapped" in the feather during the process of rapid growth and keratinization. Similar reasoning could be applied to taurine.

Discussion. Our work confirms the report of Gordon and Sizer(1) on the ability of sodium sulfate to stimulate the growth of the chicken, but differs with their observations on feathering. These workers reported that feathering of chickens fed a low-sulfur diet was poor and was improved by addition of sulfate. In our experience, the feather development of chickens fed a low-sulfur diet was satisfactory and showed no improvement when sulfate was added to the diet. Sulfate sulfur was not utilized for synthesis of sulfur amino acids in amounts large enough to suggest nutritional significance, but was incorporated into tissues chiefly as protein-bound sulfate. The growth response from feeding sulfate therefore may be due to a physiological requirement for sulfate *per se*. This requirement can evidently be satisfied by dietary sulfate or by sulfate furnished by the oxidation of the sulfur amino acids. Thus sulfate may "spare" the methionine or cystine which would be oxidized to provide the sulfate necessary for the growth of the chicken. The sulfur amino acid requirements have been determined with basal diets containing varying amounts of sulfate sulfur. Therefore, on the basis of data now available, it is impossible to estimate to what extent sulfate sulfur can replace methionine or cystine sulfur. After feeding of $Na_2S^{35}O_4$, over 20% of the S^{35} was recovered in the chickens as taurine and what are probably taurine metabolites. Taurine accounts for a substantial part (6.7%) of the total sulfur in the chicken and it is quite widely distributed in the tissues. It is possible that free taurine has some function other than as component of bile acids(6) and therefore dietary sulfate might also be of value as a precursor of taurine.

Summary. 1. Addition of 0.5% sodium sulfate to a diet low in sulfur stimulated growth of the young chicken. 2. When $Na_2S^{35}O_4$ was fed for a period of 1 week, approximately 23% of the S^{35} ingested was incorporated into the tissues. Most of the S^{35} was recovered as sulfate-S^{35}. However, about 19% was present as taurine-S^{35}, 6.6% as unknown organic compounds and barely detectable amounts as sulfur amino acids. Two weeks after $Na_2S^{35}O_4$ feeding a larger proportion of S^{35} was present in the carcasses as taurine and unidentified organic compounds and 1.5% of the S^{35} in the chickens was accounted for as cystine and methionine. It was concluded that there is a physiological requirement for sulfate *per se* and that dietary sulfate promotes growth primarily by providing sulfur for this purpose. 3. Taurine was found to be the major sulfur constituent of the water-soluble portion of feathers.

1. Gordon, R. S., and Sizer, I. W., *Science,* 1955, v122, 1270.

2. Machlin, L. J., Jackson, J. T., Lankenau, A. H., and Pearson, P B., *Poultry Sci.,* 1954, v33, 234.

3. Machlin, L. J., Pearson, P. B., Denton, C. A., and Bird, H. R., *J. Biol. Chem.,* 1953, v205, 213.

4. Machlin, L. J., Pearson, P. B., and Denton, C. A., *ibid.,* 1955, v212, 469.

5. Machlin, L. J., Struglia, L., and Pearson, P. B., *Arch. Biochem. Biophys.,* 1955, v59, 326.

6. Bolliger, A., and Gross, R., *Australian J. Exp. Biol. Med. Sci.,* 1952, v30, 181, 399.

Received July 18, 1956. P.S.E.B.M., 1956, v93.