ing is uncertain. It may mean that an excretion maximum exists for each metabolite, but further study is necessary to test this hypothesis. It is apparent from the data that calculation of a true BSP transport maximum is feasible from measurement of total color, percentage distribution of chromatographic fractions and their extinction coefficients.

Summary. During administration of a constant infusion of BSP in the dog and man, 3 chromatographically distinct metabolites were demonstrated in plasma and bile in addition to BSP itself. Part of the metabolic change involves conjugation of BSP or some BSP derivative with one or more amino acids. Some of the physiologic properties of BSP metabolites are described and discussed, particularly with reference to problems of BSP transport from plasma to bile.

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Feather Depigmentation Resulting from Feeding Molybdenum Plus Thiosulfate.* (24564)

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Achromatosis of feathers has been observed in chicks due to deficiency of lysine(1), pantothenic acid(2), and folic acid(3). Achromatosis of the hair of rats resulting from copper deficiency could be corrected by additional dietary calcium pantothenate(4). Although excess dietary molybdenum caused achromatosis of hair in cattle, the literature did not reveal reports of feather depigmentation in chickens due to excess dietary molybdenum. In studies of Miller *et al.*(5) on effect of sulfur containing compounds in alleviation of a molybdenum-induced growth inhibition, it was observed that addition of sodium thiosulfate to a diet containing 1500 ppm molybdenum resulted in feather depigmentation. The data presented here show that feather depigmentation resulting from feeding molybdenum and thiosulfate can be prevented by addition of copper to the diet.

Methods. One-week-old New Hampshire X Barred Plymouth Rock female chicks (10/group) were used except for experiment in which sodium thiosulfate-S^{35} was fed to day-old chicks. The chicks were housed in electrically heated brooders with raised wire floors, and fed the experimental diet for 3 weeks. Feed and water were supplied *ad lib.* Sodium molybdate dihydrate and sodium thiosulfate pentahydrate supplemented the basal diet (Table I). Molybdenum and copper content of basal diet and liver tissues of chicks was determined. The method of Evans *et al.*(6) was used for molybdenum determination and that of Andrus(7) for copper determination. Bone ash was determined by

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TABLE I. Composition of Basal Diet G11.

Ingredients	%
Gr. yellow corn	59.7
Soybean oil meal (44% protein)	35.0
Bone meal	3.0
Salt (4% $MnSO_4$)	.5
Choline chloride supplement (25% choline chloride)	.15
DL-methionine	.20
Vit. B_{12} supplement (10 mg/lb)	.10
A and D feeding oil (2200 A 600 D/g)	.30
	mg/kg
3 Nitro-4 hydroxophenyl arsonic acid	50
Folic acid	2
Riboflavin	4
Niacin	6
Ca. pantothenate	2
Menadione	2
Dry vit. E (44 IU/g)	600
Chlortetracycline	20

the method of Assn. of Official Agricultural Chemists, 6th Ed.(8). The basal diet contained 3 ppm molybdenum and 13 ppm copper. Radioactivity was determined on duplicate samples using methods of Kulwich *et al.* (10). In one trial sodium thiosulfate-S^{35} (outer sulfur labeled with S^{35}) was added to experimental diets. Each group contained 6 New Hampshire X Barred Plymouth Rock female day-old chicks. Sodium thiosulfate-S^{35} (0.5 mc/kg of diet) was added to diet of groups receiving basal diet, basal diet plus 1.74% sodium thiosulfate · 5 H_2O plus 500 ppm molybdenum. At the end of first week (before feather depigmentation occurred) 2 chicks from each group were sacrificed and S^{35} content of various tissues determined. At end of third week the remaining chicks were sacrificed and sulfur^{35} content of various tissues determined. Autoradiograms were made with wing feathers of groups receiving labeled sodium thiosulfate. Liver samples were hydrolyzed with 3 N HCl for 10 hours. The hydrolysates were fractionated using 0.9 X 100 cm columns of Dowex 50 resin in the hydrogen form(9). After level of radioactivity was determined on fractions obtained from ion exchange columns, the fractions containing radioactivity were concentrated and used for paper chromatography. Methyl ethyl ketone, propionic acid and water (75:25:30), tert-butanol, formic acid and water (75:15:10) and N-butanol, acetic acid and water (40:10:50) were the solvents used to develop the ascending chromatograms.

Results. Supplementation of basal diet with either molybdenum or thiosulfate individually did not cause feather depigmentation. Feather depigmentation caused by feeding molybdenum plus thiosulfate was not prevented by addition of folic acid, calcium pantothenate, or thiamine to the diet (Table II). Addition of lysine to the diet was also ineffective in preventing feather depigmentation caused by feeding molybdenum plus thiosulfate. Feather depigmentation could be prevented by addition of 50 ppm of copper to the diet. When the diet was supplemented with 1.74% sodium thiosulfate · 5 H_2O, as little as

TABLE II. Feather Depigmentation in Chicks.

Group	Treatment	No. of chicks in each group showing feather depigmentation	
		Exp. 1	Exp. 2
1	None	0 (10)†	0 (10)
2	1500 ppm molybdenum	0 (10)	
3	1.74% sodium thiosulfate · 5 H_2O		0 "
4	1500 ppm molybdenum + 1.74% sodium thiosulfate · 5 H_2O	9 (10)	9 "
5	1500 ppm molybdenum + 0.45% sulfur		0 "
6	Same as 4 + folic acid (40 mg/kg diet)		9 "
7	*Idem* 50 ppm copper*		0 "
8	" ca. pantothenate (200 mg/kg) + thiamine HCl (60 mg/kg)		7 (8)
9	" 1% L lysine HCl		8 (9)

* Supplied as cupric carbonate.

† Figure in parenthesis gives No. of chicks remaining in each group when feather depigmentation occurred.

TABLE III. Effect of Varying Level of Molybdenum and Thiosulfate in Diet on Feather Depigmentation of Chicks.

Treatment	No. of chicks in each group showing feather depigmentation
None	0 (10)*
1.74% sodium thiosulfate • 5 H_2O	0 "
250 ppm molybdenum + 1.74% sodium thiosulfate • 5 H_2O	8 (8)
500 *idem*	6 "
1000 "	8 "
1500 "	7 (7)
1500 ppm molybdenum + .35% *idem*	1 (10)
Idem .70% "	3 "
" 1.05% "	3 (9)
" 1.40% "	7 (8)

* Figure in parenthesis gives No. of Chicks remaining in each group when feather depigmentation occurred.

250 ppm molybdenum would cause feather depigmentation (Table III). However, if the level of sodium thiosulfate • 5 H_2O added to diet was less than 1.74%, then addition of 500 to 1500 ppm molybdenum to the diet was required to produce feather depigmentation.

The results of copper and molybdenum determinations on liver tissue are given in Table IV. The chicks having feather depigmentation caused by molybdenum and thiosulfate had a higher copper and molybdenum liver storage than either the basal group or the group supplemented with molybdenum. Feather depigmentation in these experiments is shown in Fig. 1. An autoradiogram of normal and depigmented wing feathers is shown in Fig. 2. Feathers in top row were from chicks receiving thiosulfate. Feathers in bottom row were from chicks receiving molybdenum plus thiosulfate. Areas on feathers lacking pigmentation correspond to the light areas in feathers in bottom row of the autoradiogram. Distribution of S^{35} in tissues of chicks sacrificed at end of third week is shown in Table V. Distribution of S^{35} in tissues of chicks sacrificed at end of first week was similar to distribution of S^{35} at end of third week.

FIG. 1. Feather depigmentation caused by high levels of molybdenum and thiosulfate in the diet.

Discussion. Although feeding molybdenum or thiosulfate will not produce achromatosis of feathers when fed individually, feather depigmentation occurs when molybdenum and thiosulfate are fed jointly. To eliminate the possibility that depigmentation in these experiments was due to a genetic characteristic of crossbred chicks, other colored breeds were fed molybdenum plus thiosulfate. Feather depigmentation was observed in Minorca, New Hampshire, Australorp and Ancona chicks fed molybdenum plus thiosulfate.

Feather depigmentation did not appear until chicks were fed molybdenum plus thiosul-

TABLE IV. Effect of Dietary Molybdenum and Thiosulfate on Molybdenum and Copper Storage in Liver Tissues.

Treatment		Liver tissues (dry wt)*			
		Molybdenum		Copper	
ppm molybdenum	Sodium thiosulfate	Exp. 1	Exp. 3	Exp. 1	Exp. 3
None		9 ± 5	.8	14 ± 2	19 ± 2
1500		25 ± 6		24 ± 12	
	1.74% • 5 H_2O		.7		16 ± 3
250	"		8.0 ± 2		34 ± 17
500	"		9.0 ± 2		30 ± 8.0
1000	"		13.0 ± 3		34 ± 5
1500	"	36 ± 5	17 ± 3	46 ± 10	40 ± 13

* Each value represents avg and stand. dev. for molybdenum and copper determinations made on 5 chicks.

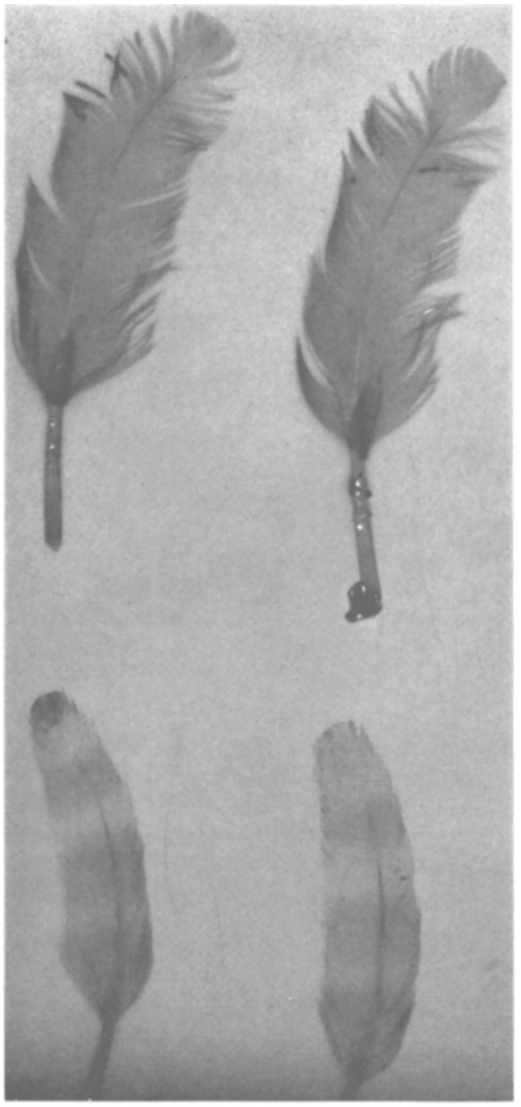

FIG. 2. Autoradiogram of feathers of chicks fed sodium thiosulfate-S^{35}. Top row: Thiosulfate only. Bottom row: Thiosulfate and molybdenum.

fate for one week. Depigmentation was most pronounced in the wings. As experimental period increased, depigmentation was seen in all feathers (Fig. 1). It has been shown that copper is essential for normal pigmentation in mammals. However, when molybdenum and thiosulfate were fed, the livers of chicks showing feather depigmentation had a greater copper content than chicks having normal feather pigmentation. Although chicks showing feather depigmentation had adequate stores of copper in the liver, it would appear that this copper was not available to the chick since addition of copper to the diet prevented feather depigmentation. It has been shown that substances that combine with copper or certain reducing substances like ascorbic acid will prevent tyrosinase from forming melanin from tyrosine(11). When feather depigmentation was first noted with diets containing molybdenum plus thiosulfate, it was suspected that the thiosulfate was either forming a copper complex or inhibiting the tyrosinase by its properties as a reducing agent. However, feeding thiosulfate without molybdenum did not cause feather depigmentation. Furthermore, addition of 1% stannous chloride to the diet containing 1500 ppm molybdenum did not cause feather depigmentation. Addition of 1% ethylenediaminetetraacetic acid (EDTA) to a diet containing 500 ppm molybdenum reduced the growth rate by approximately 25% at 4 weeks of age but did not cause feather depigmentation.

The heart, liver and kidney tissues of chicks receiving molybdenum plus thiosulfate contained more radioactivity than either the basal group or the group receiving supplementary thiosulfate (Table V). Since the group hav-

TABLE V. Distribution of $Sulfur^{35}$ in Chicks after Feeding Sodium Thiosulfate-S^{35} for a 3-Week Period.

	% dose × 10^2/g wet tissue								
	Heart	Liver	Spleen	Kidney	Gall bladder	Skin	Thigh muscle	Blood cells*	† Plasma
Basal diet	11.9	2.9	7.5	2.0	5.8	2.4	5.4	7.9	1.1
" + 1.74% Na thio sulfate · 5 H_2O	2.3	1.1	2.1	.7	1.9	.6	1.3	2.2	.5
Idem + 500 ppm molyb denum	53.8	27.4	4.4	22.0	3.5	1.4	3.9	4.9	.6

* % dose × 10^2/ml packed cell vol. † % dose × 10^2/ml plasma.

ing feather depigmentation had a higher tissue retention of copper, molybdenum and sulfur-S^{35} than the basal or the group receiving thiosulfate, it would appear that feather depigmentation was caused by copper-molybdenum-thiosulfate complex which renders the copper in tissues unavailable to the animal. Although chicks fed molybdenum plus thiosulfate, retained more of the radioactive sulfur than the other 2 groups, conversion of thiosulfate-S^{35} to the organic form was similar in all 3 groups. All of the sulfur^{35} incorporated into organic material was found in the taurine fraction. No radioactivity was found in methionine or cystine fractions. In the 3 solvent systems used for paper chromatography of fractions obtained from the ion exchange columns, the taurine fraction had RF values similar to the RF values obtained with pure taurine. After developing paper chromatograms with ninhydrin, the paper strips were scanned for radioactivity. All radioactivity was found in the spot identified as taurine. Machlin and Pearson(12) reported that the chicken can convert sulfate sulfur^{35} to taurine, methionine and cystine. They reported that most of the sulfur^{35} found in organic form was isolated as taurine, whereas only a small amount was found in methionine or cystine. While it has been shown that taurine is involved in synthesis of taurocholic acid(13), the physiological significance of taurine for any other function is not known.

No differences were noted in hemoglobin values or erythrocyte counts on blood obtained from chicks having normal and abnormal pigmented feathers. Although many chicks fed molybdenum plus thiosulfate had leg deformities, the values for bone ash of this group were similar to bone ash values obtained for the basal group and the group fed thiosulfate. Possibly manganese is also rendered unavailable to the chick when molybdenum plus thiosulfate is added to the diet.

Summary. These experiments show that addition of molybdenum plus thiosulfate will cause feather depigmentation in growing chicks. Supplementation of the diet with vitamins or lysine did not prevent feather depigmentation when the diet contained molybdenum and thiosulfate. No feather depigmentation occurred if copper (50 ppm) was added to the diet. Chicks having feather depigmentation had higher copper, molybdenum and sulfur^{35} content in liver tissues than the basal group or groups receiving supplementary thiosulfate or molybdenum. In all groups fed radioactive sodium thiosulfate, radioactivity was found in the taurine fraction but not in methionine or cystine fraction of liver hydrolysates.

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