were solely the result of higher corticosterone secretion, the thymus would be expected to be smaller in the gentled animals. The different response to gentling of rats bearing regenerating adrenals from that of intact animals probably relates to some disruption of the CNS-pituitary axis as a result of the enucleation procedure and the subsequently altered pattern of steroid secretion.

The statement of Ruegamer *et al.*(11) and Weininger(7) that gentled rats did not eat more than non-gentled controls could be confirmed for the adrenal-enucleated rats only. In our experience gentled intact animals ate somewhat more than non-gentled controls throughout the experiment, a fact which may have contributed to their slightly better growth.

Summary. 1. Gentling acts as a stressor and increases blood pressure in adrenal-enucleated as well as intact rats. 2. At the same time gentling seems to promote growth, a paradoxical situation since growth is generally considered to be depressed by the activities of ACTH and glucocorticoids. 3. This paradox may be explained by one of the currently held concepts of the mechanism of adrenal-regeneration hypertension, namely the secretion of a hypertensive steroid other than corticosterone which is, however, ACTH-dependent.

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Assimilation of Sulfur Compounds by *Pasteurella multocida.** (28543)

I. S. M. DE ISSALY† AND A. O. M. STOPPANI
Institute of Biochemistry, School of Medicine, University of Buenos Aires and Laboratory for Cell Metabolism, National Atomic Energy Commission.

Sulfur assimilation is an essential step for biosynthesis of cystine and methionine in microorganisms, and many species are able to utilize sulfate as a sulfur source(1). Some strains (virulent and avirulent) of *Pasteurella pestis* have an absolute requirement for cystine and methionine while other strains (A1122 and the "spontaneous mutant") do not assimilate sulfate, although less oxidized sulfur compounds such as thiosulfate, sulfite or sulfide can satisfy all the sulfur requirements of the "spontaneous" mutant or are used for synthesis of cysteine (cystine) by strain A1122(2). The pattern of utilization of inorganic sulfur compounds by another species of *Pasteurella (P. multocida)* is summarized below.

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Materials and methods. *P. multocida* (strain Beaufort No. 28, from Inst. Pasteur, Paris) was grown in a liquid medium consisting of meat extract (Bovril), 5 g; peptone (Biochem), 30 g; yeast extract (Difco), 1 g; glucose, 20 g; NaCl, 5 g and distilled water to 1l; pH, 7.2. After 16 hours' incubation at 37°, the liquid cultures were used to inoculate 150 ml of medium solidified with agar (25 g/l) in Roux bottles and further incubated at 37° for 24 hours. The colonies were suspended in 0.85% NaCl and collected by centrifugation at 13000 *g*, three times. The concentration of the cell suspensions was determined by comparison of the optical density with a standard curve made with a cell suspension the concentration of which was known from measurement of the dry weight (at 100°). Radioactive sulfur compounds were obtained from the Radiochemical Centre, Amersham, England, with a specific activity of 2.0 mC/mmole.

After incubation in Erlenmeyer flasks as described in Table I, duplicate 1 ml samples of the cell suspensions were washed twice in the centrifuge with 1 ml of 0.85% NaCl solution, at 4°. The cell pellets were added to 1 ml of distilled water and thoroughly mixed with 9 volumes of methanol. Total S^{35} fixed was measured by plating 300 μl of the cell suspension on aluminum planchets and counting with an end-window Geiger-Muller tube. The suspensions were then centrifuged at 4° and S^{35} activity in the supernatant (S^{35} soluble fraction) measured as above. The methanolic extracts were brought to a small volume *in vacuo* in the cold, chromatoraphed bidimensionally on Whatman No. 4 paper with the phenol-water, butanol-propionic acid solvent systems(3) and radioautographed on X-ray film. The radioactive substances were counted directly in the chromatograms with a Scott tube and identified by cochromatography with pure specimens and their R_f values in the solvent systems used (cysteine and cystathionine). Sulfate areas in the chromatograms were eluted, the sulfate precipitated as barium sulfate, filtered and counted. Total radioactivity in each substance, calculated according to Stoppani *et al.*(4), is expressed as counts/min/mg of cells.

Results and discussion. *P. multocida* incorporates S^{35} from all the precursors included in Table I. Without glucose, the apparent incorporation of S^{35} with sulfate is nearly double that obtained with sulfide, sulfite and thiosulfate. Glucose increases S^{35} incorporation with all the sulfur compounds assayed, and with glucose, the highest specific activity of S^{35} in the cells is obtained with sulfite, the next highest with sulfate. With sulfide, sulfite and thiosulfate most of the activity (59-77%) of the methanolic fraction is found in cysteine plus the related aminoacids (cystine, cysteic acid and taurine). The percentage of S^{35} in the cysteine group is increased by glucose to 87-93% of total in the S^{35} soluble fraction. Furthermore, with sulfite as substrate, homocysteine, homocystine and homocysteic acid appear radioactive in a significant proportion. Glutathione incorporates from S^{35} sulfide and sulfite which implies that the newly formed cysteine may be used for peptide and protein synthesis. On the other hand, a completely different distribution pattern of S^{35} is observed with sulfate since most of the label remains as free ion and S^{35} incorporation into the aminoacids of the soluble fraction is negligible.

Sulfur aminoacids are subject to various changes, including oxidation and degradation during paper chromatography(5). The chromatograms obtained in the experiment summarized in Table I showed a streaked radioactive area near the origin which could not be resolved nor identified. This accounts for most of the difference between total S^{35} activity in the soluble fraction and the sum of activities in the amino acids listed in Table I. Therefore, the extent of aminoacid alteration was undetermined, which prevented a reliable estimate of the relative distribution of radioactivity in the sulfur compounds identified. Nevertheless, the significant labeling of cysteine and related compounds seems to be consistent with the function of the former as an early product of incorporation of sulfide and thiosulfate through reversal of the serine-sulfhydrase reaction that occurs in

TABLE I. Assimilation of Sulfur Compounds by *P. multocida*. *P. multocida* (25 mg) suspended in mM phosphate buffer, pH 7.2 with additions as indicated. 7.7 mM sulfur compounds (total S^{35} activity: 11.1×10^7 cts./min.). 5 mM glucose. Final vol.: 3.0 ml. Incubation at 37° for 60 min.

Additions	Total S^{35} fixed	Distribution of S^{35} (cts/min./mg of cells)										
		Soluble fraction (total)	Soluble fraction									
			Cysteine	Cystine	Cysteic acid	Taurine	Cystathionine	Homocysteine	Homocystine	Homocysteic acid	Glutathione	$SO^{=}_4$
Na_2S^{35}	4475	3194	750	40	1280	21	512	0	0	13	106	0
Na_2S^{35} + glucose	5195	3132	562	28	2100	31	344	0	0	25	15	0
$Na_2S^{35}O_3$	4109	3750	0	11	2100	112	214	37	142	49	26	0
$Na_2S^{35}O_3$ + glucose	12575	8990	0	358	7550	430	243	81	234	117	63	0
$Na_2S_2{}^{35}O_3(S^{35}H)$	4421	3324	870	0	1680	13	285	0	0	0	0	0
$Na_2S_2{}^{35}O_3(S^{35}H)$ + glucose	7156	5494	930	0	4050	55	460	0	0	0	0	0
$Na_2S^{35}O_4$	8123	4596	5	0	28	5	0	0	0	0	0	4570
$Na_2S^{35}O_4$ + glucose	9467	6294	19	0	19	6	6	0	0	0	0	6280

yeast(6). In this connection it should be recalled that production of H_2S is characteristic of all the *Pasteurella* species(7). Sulfite incorporation could be explained by reduction to sulfide(8) or through cysteine sulfinate (9) although this possibility can be questioned since the reversibility of the essential step of the overall reaction (sulfinylpyruvate decomposition into sulfite and pyruvate) is not generally accepted(10). The appearance of S^{35} in cystationine and the homocysteine group, which are intermediates in the synthesis of methionine, constitutes a significant difference from strain A1122 of *P. pestis*(2) which apparently lacks the metabolic mechanism for sulfur transfer to cystathionine.

Summary. *P. multocida* utilizes sulfide, sulfite and thiosulfate (SH) for synthesis of cysteine, cystathionine, homocysteine and related aminoacids. Sulfate is not utilized for this purpose.

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