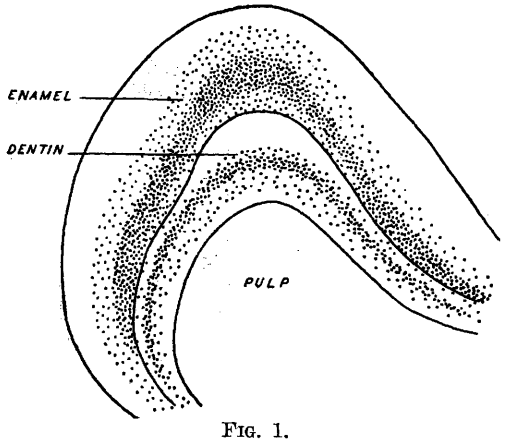

FIG. 1.
Inverted autograph of a section of a molar cusp of an 8-day-old hamster injected with P^{32} 4 days previously. The dentin shows an autographic reaction as a rather narrow dark line of granules in its mid-portion. This line moved from the inner edge of the dentin (1 day after injection) to its present position. The layer of enamel shows a reaction as a broad, dark line of granules at a distance from the ameloblasts and the dentino-enamel junction.

liable to lose their label by exchange. It was concluded that dentin grows by continuous apposition of layers of insoluble phosphate in the area close to the odontoblasts,—a process similar to the formation of periosteal and endosteal bone(6).

The deposition of phosphate in young enamel occurred at a rather considerable distance from the ameloblasts, the reaction being spread over a much broader line than that of the dentin (Fig. 1). The distance between the reaction line and the ameloblasts appeared to increase with time. The reaction did not significantly decrease in intensity with time and, therefore, was attributed to newly formed enamel crystals. Parallel studies with the von Kossa stain for calcium phosphate revealed a progressive increase in calcification from the outer (ameloblastic) limit to the inner (dentinal) limit of the enamel; there was an area of maximum calcification near the apex of the dentino-enamel junction. The radio-phosphate was deposited with a similar gradient, which, however, did not extend as far inwards as the area of maximum calcification. These findings were in agreement with the concept that the formation of enamel is due to the elaboration by the ameloblasts of an organic matrix, which is mineralized much later(7).

The results gave no information as to why the crystals of dentin and enamel form in the indicated areas.

6. Leblond, C. P., Wilkinson, G. W., Bélanger, L. P., and Robichon, J., *Am. J. Anat.*, 1950, in press

7. Chase, S. W., *J. Am. Dent. A.*, 1932, v19, 1275.

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Synthesis of Sulfur Amino Acids from Inorganic Sulfate by Ruminants.* (17693)

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The conversion of nitrogen of simple compounds in the diet to body protein of the ruminant, probably through the mediation of the micro-organisms of the paunch, is now generally accepted(1,2). However, there have

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1. Mitchell, H. H., and Hamilton, T. S., The Biochemistry of the Amino Acids., Chemical Catalogue Co., New York, 1929.

TABLE I.
Radioactivity of Milk Samples After Feeding $Na_2S^{35}O_4$.

Day No.	Time of collection	Activity in 1 ml milk in counts/min.	Activity in total collection counts/min., ×1000
1	P.M.	21	63
2	A.M.	73	370
2	P.M.	92	204
3	A.M.	82	317
3	P.M.	54	118
4	A.M.	50	105
4	P.M.	44	84
5	A.M.	45	145
5	P.M.	29	71
6	A.M.	34	107
6	P.M.	28	54
7	A.M.	27	62
7	P.M.	21	39
8	A.M.	22	54
8	P.M.	17	30
9	A.M.	14	45
10	,,	18	42
11	,,	24	56
12	,,	14	31
13	,,	11	21
20	P.M.	10	14
27	A.M.	20	27

not been any reports in the literature, to our knowledge, pertaining to the conversion of sulfate sulfur to cystine and methionine sulfur of tissue proteins. The experiments described below report evidence of this conversion.

Experimental. Approximately 9 millicuries of S^{35} in the form of $Na_2S^{35}O_4$ were fed in divided doses to a Holstein cow weighing approximately 1300 lb over the course of 8 hours. The milk was collected and the activity was measured on an aliquot as follows: 1 ml of each sample was pipetted into a planchet and evaporated under a "heat lamp" to dryness, then counted in a G-M counter with less than 2 mg mica window counter of 5 sq cm area. In all cases, the background counts were made before and after each determination of activity, and subtracted from the results obtained on the samples. The results in Table I are expressed in counts per minute of the total quantity of milk collected for each sample. A few casual samples of urine were collected, and these had the following activity in counts per minute per ml : sample I, collected 2 hours after feeding the radioactive sulfate, 463; sample II, collected 8 hours after feeding, 973; sample III, collected 5 days after feeding, 194. The proteins were isolated from milk collected on the following days: 2nd morning; 3rd morning; 11th morning; 12th morning. Ten grams of trichloracetic acid dissolved in 10 ml of water were added to 100 ml of milk from each of these days. The precipitates were allowed to form in the cold and they were then removed by centrifugation. The protein precipitates were washed very thoroughly five times with cold 2% solution of trichloracetic acid. The filtrates and washings were combined, concentrated to 100 ml and the activity was determined. The acid-washed protein precipitates were extracted 3 times with hot acetone and once with ether. The proteins were dried under a "heat

TABLE II.
Radioactivity of Milk Proteins and Protein-free Filtrates Counts Per Minute.

Day No.	Activity in milk protein from 100 ml of milk	Activity in protein-free filtrate from 100 ml of milk
2	6200	5800
3	7200	3100
11	710	<100
12	860	<100

2. E. I. du Pont de Nemours and Co., Inc., Review of Nutritional Research on Urea as a Supplementary Source of Protein for Ruminants, Wilmington, Del., 1949.

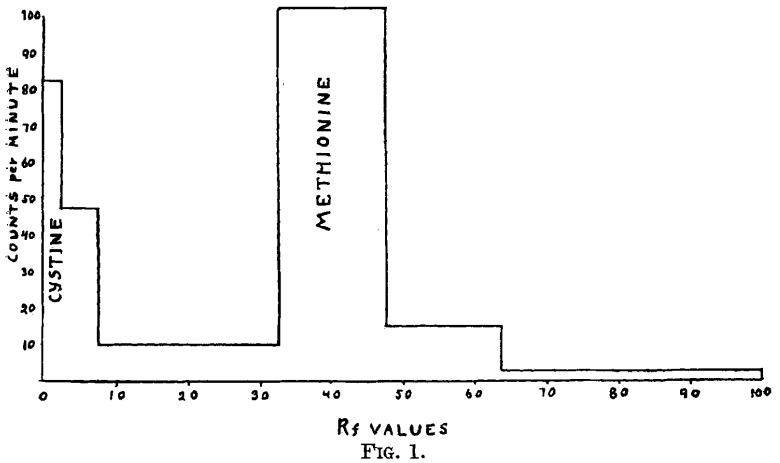

FIG. 1.
Radio paper chromatogram of 100 mg milk proteins hydrolysate.

lamp". The radioactivity of the filtrates and the proteins is recorded in Table II. One hundred milligrams of the sample of milk proteins which show the highest activity (third morning) were hydrolyzed with 10 ml of 6 N HCl under reflux for 24 hours. The hydrolysate was decolorized with 100 mg of carbon and the excess of HCl was removed by evaporation to dryness at 35° *in vacuo* and repeating the process on addition of water 3 times. The dry residue was then taken up in 5 ml of water. One-hundredth ml aliquots of the hydrolysate, 12 mm apart, were put on 5 sheets (18 x 21 inches) of S. and S. No. 598 filter paper until the entire 5 ml of the hydrolysate were applied. Cystine, methionine, and $Na_2S^{35}O_4$ solutions were also applied to each sheet for control purposes. The chromatograms were developed with water-saturated N butanol-acetic acid (10 $^{v}/_{v}$ for 24 hours (3). Control strips cut from the sheets indicated that the sulfate does not move from the point of application with this solvent† Cystine had an Rf of 0.04 (0.03-0.05) and methionine an Rf value of 0.41 (0.39-0.43). In view of these findings, the sheets were cut as follows: Band I, Rf 0.00-0.03; Band 2, Rf 0.03-0.05; Band 3, Rf 0.08-0.33; Band 4, Rf 0.33-0.47; Band 5, Rf 0.47-0.64; and Band 6, Rf 0.64-1.00. The analogous bands from each of the 5 sheets were thoroughly extracted with hot water, the extracts were concentrated to dryness, and the activity of each extract was determined as described above. The results are summarized in Fig. 1.

Results. Our data indicate that sulfate sulfur is converted in appreciable quantities to cystine and methionine sulfur when fed to a ruminant. It has been previously shown(4) that even non-ruminants are able to exchange a small quantity of sodium sulfide sulfur for the cystine sulfur of their tissue proteins. However, there are no experiments which show that sulfate sulfur will function in this manner. As far as we are aware, no evidence has been presented which indicates the synthesis of methionine from inorganic sulfate, although this is known to be true in the case of many micro-organisms. Whether the synthesis of cystine and methionine from inorganic sulfate proceeded via two independent paths or whether the cystine originated from the synthesized methionine or *vice versa* in our cow is not possible to assess from the data at hand.

Summary. Radioactive sodium sulfate was fed to a cow, milk was collected for several days, and the proteins were isolated. The

3. Block, R. J., and Bolling, D., The Amino Acid Composition of Proteins and Foods, 2nd Edition, C. C. Thomas, Springfield, Ill., 1950.

† The $BaSO_4$ precipitate from the hydrolysate of 422 mg of milk proteins (third morning) gave only 30 counts per minute.

4. Dziewiatkowski, D. D., *J. Biol. Chem.*, 1946, v164, 165.

proteins showed appreciable radioactivity. From the hydrolysate of the radioactive proteins cystine and methionine were separated on chromatograms, and eluted. Both cystine and methionine contained radioactive sulfur in appreciable amounts.

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Relative Resistance of Newborn Mice to Poliomyelitis Virus.* (17694)

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The present study was begun with the hope that propagation of the Lansing strain of poliomyelitis virus in newborn mice might yield material with higher virus titers. It appeared probable that this might be achieved, because repeated observations in this laboratory(1) indicated that when the mouse-adapted Hawaii dengue virus, which also produces flaccid paralysis in intracerebrally inoculated mice, is injected in suckling mice less than one week old, the infected brain suspensions usually have virus titers, which are approximately 100 times greater than those obtained from brains of mice inoculated at 2 weeks of age or older. However, a preliminary test carried out with the Lansing virus suggested that the newborn mice may be more resistant that the older animals. Since this observation was not in accord with the recorded properties of all other neurotropic viruses in mice(2-3,4), as systematic study was carried out on larger numbers of animals to elucidate this unexpected behavior of poliomyelitis virus.

Methods. The albino mice used in these tests were obtained from a Cincinnati dealer, who could not be certain of their genetic origin or composition except that they were probably "Swiss" mice. Pregnant females obtained from the dealer several days before expected delivery of young, were placed in separate boxes and the birth of new litters was noted twice daily. Mice were not used on the day of birth. All the tests were carried out during the months of June, July and August, 1948, when the temperature in the animal quarters was not infrequently 90 to 100°F. Following inoculation the suckling mice were returned to their mothers, and each family was observed separately. The virus consisted of a large lot of 10% centrifuged mouse brain suspension in saline, aliquots of which were distributed in sealed glass ampules which were maintained in the frozen state in a "dry ice" storage box; it represented the 203rd mouse brain passage and had an LD_{50} of $1 \times 10^{-3.7}$ for the 0.03 ml dose. Since only 0.01 ml was inoculated intracerebrally in the suckling mice, the dose for the older mice, used for control in each test, was also 0.01 ml. The inoculations were carried out without anaesthesia.

Results. When 0.01 ml of the 10^{-1} virus suspension, containing approximately 170 LD_{50} (based on titration in mature mice using 0.03 ml doses), was inoculated intracerebrally in mice of different ages, the significant difference between the newborn and older mice was in the length of the incubation period (Table I) and in the survival time after onset of paralysis (Table II). With regard to prolongation of the incubation period, there was no apparent difference between suckling mice tested 1, 2 or 3 days after birth, it was still definite but progressively less marked in those tested at 4 to 7 days, while at 15 days after

* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

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2. Olitsky, P. K., Sabin, A. B., and Cox, H. R., *J. Exp. Med.*, 1936, v64, 723.

3. Casals, J., *J. Exp. Med.*, 1940, v72, 445.

4. Lennette, E. H., and Koprowski, H., *J. Immunol.*, 1944, v49, 175.