4) The arrival time of the first subpulse can be timed, in a single pulse, to an accuracy of about 2 msec. This means that the pulse period can be measured with two pulses to an accuracy of one part in 10^6 in an hour, one part in 10^7 in a day, and one part in 10^9 in a year. By the observation of many pulses in the observing period, these accuracies can be improved by perhaps two orders of magnitude.

5) The construction of models of the radio emitting object and associated physical processes must lead to a situation in which three primary subpulses, nearly equally spaced in time, must occur, but with large variations in relative subpulse intensity from pulse to pulse. Although several concepts of the object leading to two subpulses can be imagined, no schemes producing three subpulses readily present themselves.

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Beta Mercaptolactate-Cysteine Disulfide: Analog of Cystine in the Urine of a Mentally Retarded Patient

Abstract. *β-Mercaptolactate-cysteine disulfide, a hitherto undescribed analog of cystine, was isolated from the urine of a mentally retarded patient. The properties of this substance are described, and its structure is confirmed by mass spectrometry and by partial synthesis.*

Metabolic abnormalities were discovered in a number of patients in mental institutions in Massachusetts who were screened for such by the urine nitroprusside test. As judged by the results of paper chromatography of the urine, most of these patients were either homozygous or heterozygous for cystinuria. However, in the urine of one patient a heretofore undescribed amino acid was found. This patient (45 years old) was the product of a sibling mating, had no physical abnormalities, but was mentally retarded (I.Q. = 50). No excess of cystine was found in fresh urine, and the new amino acid gave a pink color with the cyanide-nitroprusside reagent.

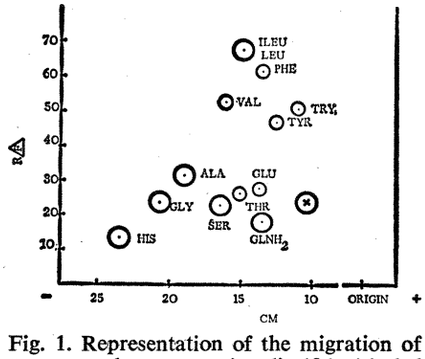
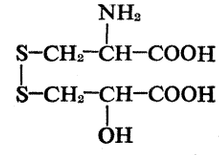


Fig. 1. Representation of the migration of β-mercaptolactate-cysteine disulfide (circled *X*). Electrophoresis in the horizontal direction was performed in 8 percent formic acid, *p*H 1.6; 3.5 kv; 170 ma; 45 minutes. Chromatography in the vertical direction in butanol: acetic acid: water (12:3:5).

The new amino acid was separated from other naturally occurring amino acids by a combination of high-voltage electrophoresis and partition chromatography (*1*) (Fig. 1). The isoelectric point of this amino acid was *p*H 2.2. Upon thin-layer chromatography (*2*) the new substance ran close to cystine. In cation-exchange chromatography (Technicon automatic amino acid analyzer) the new substance eluted between threonine and glutamic acid; the ratio of light absorption at 440 nm to that at 570 nm was a little higher than is usual for nonsulfur-containing amino acids and was comparable to that found for cystine. For isolation of the amino acid the urine was adjusted to *p*H 8 and poured onto a Dowex 2 ion-exchange column (acetate form). The column was eluted with 1*N* acetic acid and then with 4*N* acetic acid. The 4*N* acetic acid eluate was applied directly to an ion-exchange column of Dowex 50 (H^+). This column was washed first with water and then with 1*N* ammonia. The ammonia eluate was evaporated at reduced pressure. The pigmented residue was placed on a column of Bio-Gel P-2. The fractions obtained by elution with water were evaporated at reduced pressure. The active fraction was purified by high-voltage electrophoresis (Whatman 3MM paper washed in buffer: pyridine–acetic acid, 0.07*M*, *p*H 5.2). The area containing the active fraction was cut out, and the new amino acid was eluted with water. The material could be reduced with either dithiothreitol or sodium borohydride. The reduced product was treated with iodoacetic acid, and the only ninhydrin-positive substance identifiable was carboxymethylcysteine. Oxidation of the amino acid with either bromine water or performic acid yielded cysteic acid. The substance was therefore thought to be an unsymmetrical disulfide, one half being cysteine and the other half, a ninhydrin-negative substance. The new amino acid was unstable in water at neutral *p*H, and after a few days three substances could be detected on high-voltage electrophoresis with the H_2PtCl_6–KI reagent. One was the original material, one was cystine, and the other a new disulfide which we believe to be derived from the ninhydrin-negative portion of the molecule. This new disulfide did not migrate on high-voltage electrophoresis in aqueous formic acid (*p*H, 1.6) but migrated much more rapidly than the original substance toward the anode at *p*H 5.2 and was isolated after purification in this system.

Further definitive information was obtained from the mass spectra of a series of more volatile derivatives. These comprised the methyl-, propyl-, and butyltrifluoroacetyl esters of the new amino acid and the methyl-, propyl-, and butyltrifluoroacetyl esters of the ninhydrin-negative disulfide derived from it. All derivatives were prepared by the method of Gehrke and Stalling (*3*). Mass spectral measurements (Varian MAT SM1) indicated molecular ion formulas compatible with a parent structure $C_6H_{11}NO_5S_2$. The presence of two carboxyl groups was confirmed by the shift of 28 mass units between molecule ions of the propyl and butyl esters. The presence of two sulfur atoms was inferred from isotope-ratio determinations (S^{34}:S^{32}) on the molecule ion peaks in each spectrum. From the foregoing mass-spectrometry data the structure of the parent substance which is most consistent with the data is



Confirmatory evidence of the structure of the ninhydrin-negative disulfide (β-mercaptolactate disulfide) was obtained by its reduction with Raney nickel in aqueous solution at 45°C for 30 minutes. After filtration and evaporation, the residue was esterified with methanolic HCl, and the product was identified as methyl lactate by gas chromatography and mass spectrometry. Partial synthesis of the amino acid was achieved by treatment of cystine in acid solution with sodium nitrite (*4*). This reaction resulted in the replacement of one or both of the amino groups of cystine with hydroxyl groups. These hydroxylated derivatives were isolated by high-voltage electrophoresis. They were shown to have the same electrophoretic properties as the newly described sulfur-containing amino acid and the ninhydrin-negative disulfide derived from it. Acyl esters of these compounds were made and shown to have mass spectra identical to those obtained from the naturally occurring material.

The excretion of the newly found substance in the urine represents a new disorder of sulfur amino acid metabolism, a disorder which can be detected by the cyanide-nitroprusside technique. A substance of this structure could arise as the result of a defect of cysteine or cystine metabolism.

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Pineal Gland: Influence on Gonads of Male Rats Treated with Androgen Three Days after Birth

Abstract. *Either blinding or the injection of 1 milligram of testosterone propionate into male Sprague-Dawley rats, 3 days old, results in testes and accessory organs (seminal vesicles and coagulating glands) that are smaller than normal when the rats are 72 days old. The response to blinding is prevented by removal of the pineal gland, whereas the response to treatment with testosterone is unaffected by pinealectomy. Combination of the two treatments in 3-day-old rats causes testes to be less than one-third their normal size at 72 days of age; pinealectomy in these rats permits the reproductive organs to grow to the same size as those in the androgen-treated animals.*

Blinding of male and female albino rats retards the development of their reproductive organs (*1*, *2*). This effect can apparently be mediated by way of the pineal gland, since in pinealectomized, blinded rats the gonads mature normally (*2*). The mechanism whereby light deprivation and pineal substances interfere with the normal functioning of the hypothalamo-pituitary gonadal axis is unknown. However, the pineal antigonadotropic factor may act on the hypothalamus (*3*).

The combination of treatment with testosterone (when the rats were 5 days of age) and blinding of rats (when the rats were 21 days of age) was followed by failure of gonadal development until the animals are at least 75 days old (*4*). Because the pineal gland markedly influences gonadal size in golden hamsters (*5*), the ability of the gland to mediate the effects reported by Hoffmann *et al.* (*4*) was tested.

Males of 15 litters of Sprague-Dawley rats, all born within the same 3 days, were used in the experiment, which was performed from May to July. On day 3 after birth, approximately half of the males of each litter received one subcutaneous injection, between the scapulae, of 1 mg of testosterone propionate (TP) in 0.1 ml of sesame oil. All of the androgen-treated males were either pinealectomized or sham-operated, and half were blinded. Two-thirds of the untreated rats were blinded (half pinealectomized and the other half sham-operated); the rest of the animals were not treated. Operations were performed on 3-day-old animals that had been placed on ice until they were hypothermic. Rats were blinded by removal of the eyes after the optic nerve was sectioned. All animals were maintained under conditions of 12 hours of light per 24-hour period (Table 1). The litters (males and females) were placed with their respective dams and weaned at 23 days of age. At 72 days of age the males were killed, and the weights of the reproductive organs were recorded. The data in Table 1 are expressed as absolute weights, because the sizes of several organs (including the adrenal glands) were the same in all groups of animals, an indication that the observed results were a sequel of a specific inhibition of gonadotropin synthesis, release, or action, which possibly indirectly affects body growth.

The data (Table 1) show that blinding (group 2) inhibits growth of testes and accessory organs; the response is prevented by pinealectomy (group 3); this result confirms earlier observations (*2*). Likewise, treatment with TP and sham pinealectomy (group 4) interferes with the development of the male reproductive organs. This response, however, is not affected by extirpation of the pineal gland (group 5). When TP was given to blinded, sham-operated animals (group 6), the reproductive organs were much smaller than those of the other groups; thus the testes of 72-day-old animals weighed 906 mg. The accessory organs (seminal vesicles and attached coagulating glands) also were smaller. We do not know whether the fact that the testes were small in the 72-day-old rats means that there was a retardation of growth of the testes of a near-normal gonadal development and then a subsequent atrophy. The reproductive organs of TP-treated, blinded rats whose pineal glands were removed (group 7) were significantly larger ($P<.001$) than those of similarly treated, nonpinealectomized animals (group 6); they were significantly smaller than those of normal controls (group 1); and they were similar in size to those of nonblinded rats that had been treated with TP (groups 4 and 5).

On histological examination, the testes of all animals (except those of the