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

Table 1. Effects of neonatal testosterone propionate (TP) treatment, blinding, and the pineal gland on the body weights and the size of the testes and of accessory organs (seminal vesicles and coagulating glands) in male albino rats.

Animals (No.)	Body weight (g)*	Testicular weight (mg)	Accessory organ weight (mg)
	Group 1: Untreated		
8	318 ± 11	3110 ± 69	389 ± 31
	Group 2: Blinded, sham-pinealectomized		
8	254 ± 8†	2508 ± 94†	238 ± 27†
	Group 3: Blinded, pinealectomized		
8	310 ± 10	3060 ± 103	368 ± 34
	Group 4: TP-treated, sham pinealectomized		
10	301 ± 14	2589 ± 78†	291 ± 19†
	Group 5: TP-treated, pinealectomized		
9	292 ± 9	2480 ± 96†	278 ± 26†
	Group 6: TP-treated, blinded, sham pinealectomized		
9	217 ± 11†	906 ± 161‡	44 ± 12‡
	Group 7: TP-treated, blinded, pinealectomized		
8	246 ± 5†	2155 ± 79†	255 ± 23†

*Mean body and organ weights ± standard errors. †Significantly different ($P<.05$) from similar weights of untreated controls (group 1). ‡Significantly different ($P<.001$) from similar weights of untreated controls (group 1).

blinded, TP-treated animals) were, for the most part, normal in appearance. The small testes of blinded, androgen-treated rats with intact pineals exhibited a marked diminution in the number of spermatids.

The hypothalamic sites at which androgen acts to modify the cyclic release of luteinizing hormone appear to be the anterior and preoptic areas (*6*). Our results show that TP-treatment or blinding alone does not greatly retard gonadal development, although the testes are smaller than normal under both conditions. However, when the treatments are combined, the overall effect is an apparent restriction of folliculotropin synthesis or release (*4*). Possibly, the onset of gonadotropin synthesis or release is delayed or decreased, and, if animals had been maintained for longer periods of time, there would have been normal (or at least increased) spermatogenic activity and gonadal size. The specific site at which the pineal principles act is still undefined (*7*).

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Starch Accumulation in Shoot-Forming Tobacco Callus Cultures

Abstract. *Microscopic histochemical examinations of cultured tobacco callus disclosed a strong correlation between starch accumulation and shoot initiation. The accumulation started before any observable organized development and was heaviest in cells of loci which ultimately gave rise to organ primordia. Treatment of tissue cultures with gibberellin prevented starch accumulation and organ formation.*

Cultures of plant callus are useful in the exploration of factors regulating the initiation of plant organs (*1*). In contrast to the more conventional methods, in which stem cuttings are used, callus cultures offer better control of nutritional and environmental factors and permit one to distinguish between initiation of organs and their outgrowth from preexisting primordia. It has been shown with callus cultures that the basic regulatory mechanism underlying plant organ initiation involves a balance between auxin and cytokinin (*2*). Unfortunately, this mechanism cannot be demonstrated among a wide range of plant species, and attempts to induce organ initiation through treatments with auxin, cytokinin, and other morphogenic substances have been generally unsuccessful. Possibly, the elucidation of the regulatory mechanism can be more effectively accomplished if information concerning the internal physiology of the cells which participate in organogenesis becomes available.

Treatment of tobacco callus cultures with various amounts of indoleacetic acid and kinetin apparently results in correlated changes in the composition of scopoletin and its glycoside, scopolin in the tissue and in the nutrient medium (*3*). The significance of this finding remains unknown. Lee (*4*) suggested that the action of phenolic compounds, including L-tyrosine, in stimulating or inhibiting shoot initiation in tobacco callus culture lay in their ability to regulate the activity of indoleacetic acid oxidase and thus the effective concentration of indoleacetic acid in the tissue. Lee further suggested that, since tyrosine is also a precursor of lignin, some lignification of cells may be a prerequisite of organ initiation; thus, he supported the concept of Steward *et al.* (*5*). However, Dougall (*6*) showed that tyrosine was more rapidly incorporated into protein than into lignin.

We conducted a microscopic histochemical study on freeze-substituted samples of cultured tobacco (*Nicotiana tabacum* L., var. Wisconsin 38) callus. The procedure of tissue culture was a modification of one previously described (*7*). The cultures were maintained in constant darkness in one of two nutrient media, one containing 2 mg each of indoleacetic acid and kinetin per liter, and another further supplemented with these substances (in milligrams per liter): L-tyrosine, 100; adenine sulfate, 160; and NaH_2PO_4, 340. The first medium caused the development of only unorganized callus, whereas the latter promoted the initiation of large numbers of shoots. The cultures were sampled frequently during development and compared histologically and histochemically. The metabolism with respect to carbohydrates, nucleic acids, and proteins was investigated. The most dramatic difference was that of starch accumulation. The periodic acid-Schiff, aniline blue-black staining procedure was used; the presence of starch was confirmed by the use of the IKI and zinc-chlor-iodide reagents (*8*).

The callus initially showed little or no stored starch and no discernible cell division. The cells were highly vacuolated and contained inconspicuous