'small'; others showed atypical development of the head and neural structures and were classified as 'abnormal'. In view of a certain amount of variation in developmental stages and in size between littermates occurring normally, it is not possible to assign a particular genotype to the 'small' embryos.

DUNN and GLUECKSOHN-SCHOENHEIMER[6] reported that 'abnormal' embryos from crosses of lines A by 29 were smaller and less developed than their tailless littermates and that they had abnormalities involving anterior structures, e.g., microcephaly, microphthalmia, and anencephaly. In the present study of embryos between the ages of 9 and $12^1/_2$ days, 11 were found with head abnormalities, 17 with abnormal neural structures, and 5 with herniated hearts, some of the embryos possessing more than one atypical structure.

Most of the animals with abnormal head structures appeared to be microcephalic and two possessed abnormal mandibular arches. Abnormal neural structures included asymmetrical neural folds in embryos that had not developed beyond the neural fold stage. Two additional embryos had allantois which had apparently failed to join the chorion at earlier stages.

The results of this study as well as of the previous one[6] seem to indicate that interaction of t^0 and t^1 during embryogeny varies in severity. At one extreme, death of t^0t^1 compounds may occur at very early stages, as evidenced by the large number of sites of resorption found in litters of interline crosses on the 9th day of gestation. At the other extreme, some t^0t^1 compounds are viable and normal tailed at term. Between these two extremes spreads the 'abnormal' group with a wide range of abnormalities leading to death of embryos at different stages. This is in distinct contrast to the sharp phase specificity shown by embryos homozygous for either recessive allele which die at definite stages of development[3].

The same lack of sharp phase specificity of effect was reported in an investigation of a group of genetically similar lethal t alleles extracted from different populations of wild house mice, where the homozygous recessive condition of each allele led to a lethal period extending between 8–10 days[8]. Abnormal embryos showed a wide range in degree of differentiation, some developing no farther than the egg cylinder stage, while others formed extraembryonic membranes.

In our present study control embryos for comparison with those dissected from interline crosses were obtained from matings within line 29. Previous investigations had shown the t^1 allele to be a preimplantation lethal[9], but in the present study resorption sites were found for 33 of a total of 85 embryos between $8–12^1/_2$ days, indicating death of such embryos after implantation. Numerically, the proportion of resorbed embryos may well account for the t^1 homozygous class. In addition, the average number of embryonic sites of 9.4 at time of dissection appears rather high for a preimplantation lethal.

These results open the possibility that our tailless line 29 carries a new t allele (t^x) which acts as an early postimplantation lethal and which arose sometime in the past history of our Tt^1 line. Since t^x would be indistinguishable from t^1 in its interaction with T in producing taillessness, it could have been carried through one of the narrow bottlenecks that frequently develop in the course of breeding as difficult a strain as this tailless one with two lethal genes.

If the indications of the presence of a new mutation in line 29 can be confirmed, it would explain some of the differences between earlier studies by DUNN and GLUECKSOHN-SCHOENHEIMER[6], and the present investigation. Therefore, the identification of the recessive alleles involved in the crosses reported here will be the subject of an extensive developmental study.

Zusammenfassung. Mehrfach wurde gezeigt, dass zwei rezessive letale Allele in der Hausmaus (t^0 und t^1) lebensfähige normale Tiere in heterozygoter Kombination t^0/t^1 produzieren. Neuere Daten machen wahrscheinlich, dass das t^1-Allel mutativ abänderte, so dass sowohl seine homozygote Wirkung als auch die Zusammenwirkung mit t^0 beeinflusst worden ist. Hingegen sind Kombinationen von t^0 und dem neuen Allel nicht mehr lebensfähig, und Homozygote sterben erst nach der Implantation.

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STUDIORUM PROGRESSUS

The Discrimination of Various Cystine Sulfoxides

A controversy exists concerning the constitution of cystine disulfoxide. Based on infrared spectral interpretation, SWEETMAN[1] deemed it to be cysteine thiolsulfonate (I). The isomeric symmetrical disulfoxide structure (II) was advanced by LAVINE and TOENNIES[2] on the basis of its facile reduction to cystine.

LAVINE and TOENNIES[2] have reported the synthesis of cystine disulfoxide by the oxidation of cystine perchlorate with perbenzoic acid in anhydrous acetonitrile. EMILIOZZI and PICHAT[3] have used performic acid in formic acid as the oxidant. It has now been found that both procedures produce both possible isomers. The method of LAVINE and TOENNIES yields mainly the symmetrical disulfoxide, while that of EMILIOZZI and PICHAT affords as main product the thiolsulfonate. The two isomers can be distinguished by their decomposition points, the thiolsulfonate becoming partly viscous above 205°, whereas the symmetrical disulfoxide remains dry and darkens slowly up to 240°.

Both compounds possess a strong peak in the infrared at 8.93 μ. This is the same peak shown by cystine monosulfoxide (Cys(S→O)-S-Cys) (III)[4] and is responsible for the controversial structure assignments. The remaining parts of the spectra are distinctly different (Figure 2).

BREDERECK, WAGNER, BECK, and KLEIN[5] showed recently, with several aromatic sulfoxides, that the band at 1050 cm^{-1} (9.54 μ), which was assigned by SWEETMAN to the sulfoxide group, varies from 1040 cm^{-1} to 1130 cm^{-1} and that various sulfones, disulfones and disulfides exhibit

[1] B. J. SWEETMAN, Nature *183*, 744 (1959).
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sharp bands in the same region. Methionine sulfoxide exhibits a strong split band with peaks at 1033 cm^{-1} and 1019 cm^{-1}. The assignment of bands to structures including chemical reasoning seems, therefore, the more justified when chemical evidence is available for distinction between various oxidation levels. The IR-spectrum of I is in good agreement with the one given by BREDERECK et al.[5]. The bands at 711 cm^{-1} (14.1 μ) and at 1191 cm^{-1} (8.39 μ) seem to be quite specific for thiolsulfonate. The sulfoxide group has only a weak band at 14.1 μ in the aromatic as well as in the amino acid series. The double peak between 8.2–8.5 μ for all disulfides and their oxidized derivatives studied closes to a single band in the thiolsulfonate.

The unsymmetrical cystine disulfoxide (I) is practically insoluble in water and a lower pH is required to bring it into solution. The symmetrical derivative (II) is a little more soluble than cystine. The two isomers are best separated by alternately dissolving in 0.25 *N* HCl (50 mg in 10 cm^3), whereby I remains undissolved, and precipitating by adding dropwise 8 *N* ammonium hydroxide with cooling and stirring. The pH should never exceed 6 because during the titration both isomers start to decompose at pH's between 6 and 7 (see Figure 1). As reported by EMILIOZZI and PICHAT, the compounds are split to cysteine sulfinic acid.

The fission below pH 7 is of significance in the detection of the disulfoxide in samples of monosulfoxide by titration. For the infusion of the base, a commercial infusion pump[6] was used which delivers the titrant continuously at a chosen speed. An interruption in the continuity of the titration curve (Brown pH Recorder) indicated the presence of a disulfoxide. The symmetrical disulfoxide is particularly characterized by a sharper interruption point (Figure 1).

For a further characterization of the oxidation level of disulfide bonds, the electrolytic hydrogenation in a commercial desalter[7–11] was used as proposed by BENESCH and BENESCH[9] with modification by THOMSON and MARTIN[12]. Further modifications were necessary, however. The electrodes were connected with a recorder which was calibrated for ampères and the rate of escaping hydrogen tracked with the aid of a bubble counter. The cathode space was therefore closed except for the outlet for the bubbles. The mercury pool cathode was stirred by a magnet.

The insoluble disulfoxides were suspended. The end of the reduction or of a reduction step is recognizable by a rise in the bubbles/min count to an almost constant value. In simple cases the hydrogen is given by the coulombs used, as indicated by the recorder, minus the recovered equivalents of hydrogen. The result is less reliable if a large excess of hydrogen is required for the reduction.

The electrolytic hydrogenation allows a sensitive discrimination between cystine monosulfoxide (III), cysteine sulfenyl cysteine sulfinate (II), and cystine thiolsulfonate (I). Unlike the reduction with hydriodic acid which reduced the disulfoxides to cystine[2], the electrolytic hydrogenation could be conducted under mild conditions whereby the reduction steps became visible. Under these conditions, the first step in the reduction of cystine monosulfoxide (III) was the separation of the S-S linkage. Only one equivalent of cysteine was formed.

Cysteinyl cysteine sulfonate (I) also formed one equivalent of cysteine, but more than five times as many coulomb equivalents were needed than for the monosulfoxide. This is partly due to its lower solubility and partly to the steric hindrance of the hexavalent sulfur[1]. The cystine disulfoxide (II) required a larger excess of

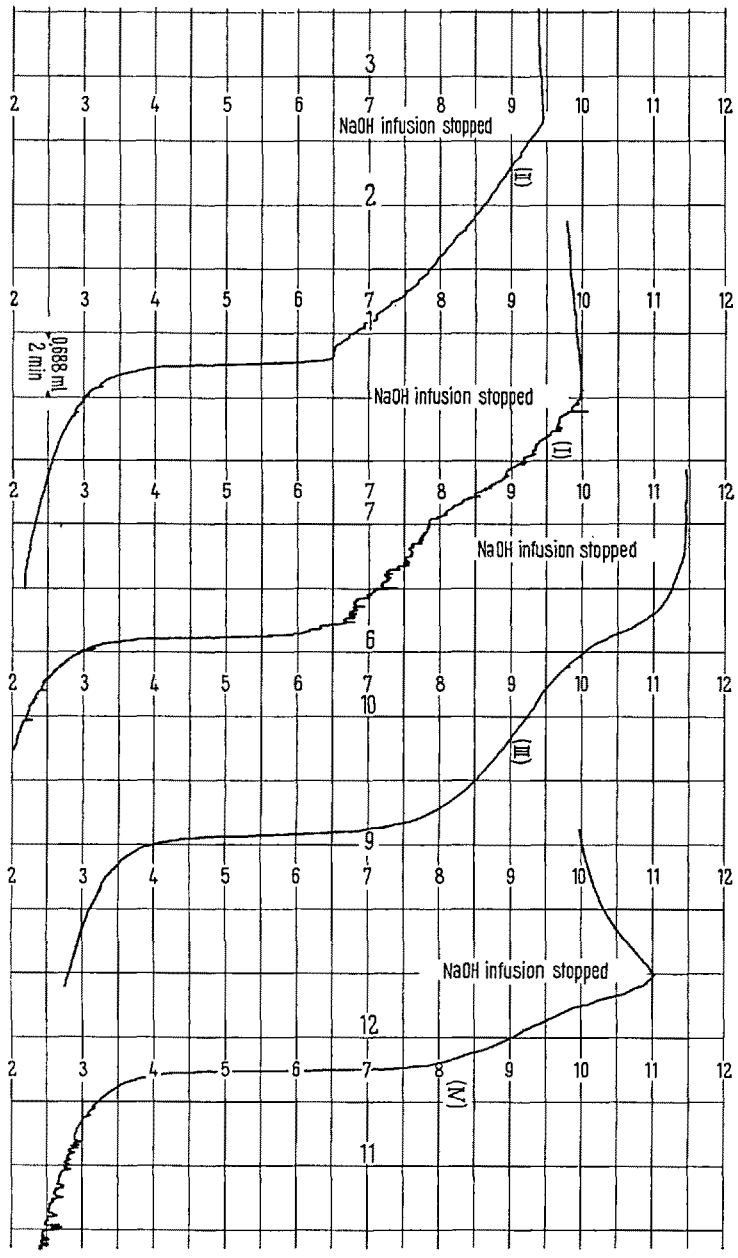

Fig. 1. Automatic titration of: (I) *L*-Cystine thiosulfonate 52 mg dissolved in 0.5 ml *N* HCl and diluted to 25 ml with H_2O. (II) *L*-Cystine disulfoxide 64 mg dissolved in 0.5 ml 1 *N* HCl and diluted to 30 ml with H_2O. (III) *L*-Cystine monosulfoxide 56 mg dissolved in 0.5 ml *N* HCl and diluted to 25 ml with H_2O. (I), (II), (III) were titrated with 0.2 *N* NaOH. (IV) *L*-Cystine 64 mg in 0.5 ml *N* HCl and diluted to 30 ml with H_2O titrated with 0.5 *N* NaOH. (I), (II), (IV) show the pH decreasing after the infusion of the titrant was stopped.

[6] Model 600–900, Harvard Apparatus Co., Dover (Mass.).
[7] From Research Specialties Co., Richmond (California).
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[12] C. G. THOMSON and H. MARTIN, Biochem. Soc. Symposia No. 17, Gluthathione, p. 17 (Univ. Press, Cambridge 1959).

hydrogen than did the thiosulfonate. Overlapping reduction steps seemed probable. At the end two equivalents of cysteine were formed.

The cysteine solution is then titrated amperometrically in tris buffer (tris-hydroxymethyl-methylamine or 2-amino-2-hydroxymethylpropane 1:3 diol). On addition of cysteine to a buffered solution of silver tris ion $Ag(tris)_2^+$ stirred with a rotating platinum electrode (see below)[13,14], the current drops very sharply and rises again slowly to a constant value, which is, however, much below the start. This drop is assumed to be caused by a catalytic reaction of cysteine with the electrode[15,16]. This drop in the current was utilized, after proper instrumental modifications, as endpoint indication in the quantitative determination of cysteine. Hydrogenolysis followed by amperometric titration was used in the estimation of sulfoxides.

The hydrolytic fission interferes also during electrolytic hydrogenation in aqueous suspension. With a labile oxide the plot of ampere versus time exhibits a steadily increasing conductivity under constant voltage.

In the amperometric titration suggested for biological microanalysis by BENESCH and BENESCH[9] and THOMSON and MARTIN[12], $AgNO_3$ is added as the titrant to a sample buffered with tris. In this titration the fading of the sharp catalytic response to cysteine overlaps with the rather poor response to added $AgNO_3$. In our estimation we add the cysteine as titrant to a chosen amount of silver tris ion $(Ag(tris)_2^+)$[17]. Every drop of cysteine causes first a catalytic response even in the presence of silver tris ions, which is immediately reversed by reaction of the supposed catalytic intermediate with silver tris ions. When all silver is titrated, the catalytic response remains. This titration therefore uses an unreversed catalytic response as the end point indication of the amperometric titration. The implication is that the other fission product, the very labile cysteine sulfenic acid, is not appreciably reduced under the same conditions and does not cause the catalytic drop used as an end point indication[17].

Zusammenfassung. *L*-Cysteinsäure-*L*-cysteinylester und symmetrisches *L*-Cystindisulfoxyd wurden als zwei isomere Oxydationsprodukte des Cystins isoliert und durch IR-Spektrum und verschiedenes Verhalten bei der elektrolytischen Reduktion charakterisiert. Mit Hilfe gleichmässiger mechanischer Titration wurden die pH-Beständigkeitsbereiche und das kritische pH beginnender Hydrolyse als abrupte Wendung in der Titrationskurve automatisch registriert.

Die katalytische Wechselwirkung des Cysteins an der rotierenden Platinelektrode wurde indirekt als Endpunktindikation der amperometrischen Titration des Cysteins in Tris-Puffer benutzt.

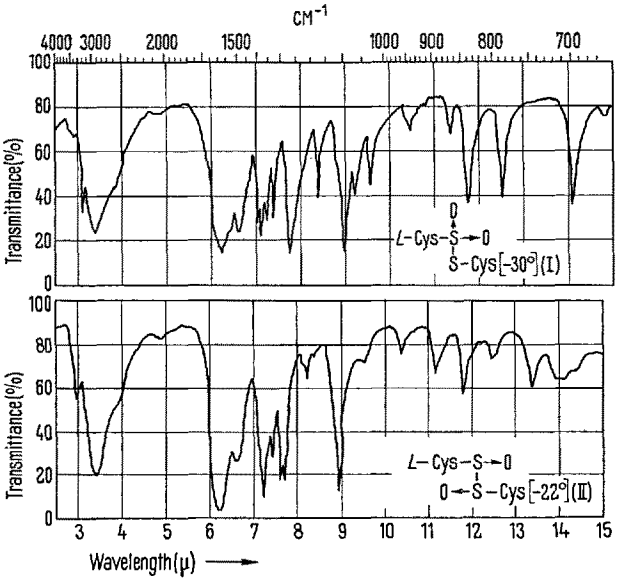

Fig. 2. IR-spectra of *L*-cystine thiolsulfonate (I) and *L*-cystine disulfoxide (II) in KBr 1:400 measured on a Perkin Elmer Spectrophotometer, Model 21, with Slave Recorder.
(I) Synonyms: Cysteinyl cysteine sulfonate, Cysteic acid cysteinyl ester, unsymmetrical cystine disulfoxide, Cystinethiolsulfonate.
(II) Cysteine sulfenyl cysteine sulfinate, symmetrical cystine disulfoxide.

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[16] In the literature the term catalytic wave of cysteine is mainly assigned to a hydrogen wave which appears in the polarogram of cysteine and of cystine in the presence of Co^{2+} ions (see W. LAMPRECHT, S. GUDBJARNASON, and H. KATZLMEIER, Z. physiol. Chem. *322*, 52 (1960)).

[17] Various silver salts of cysteine and oxidized fission products are formed during the amperometric titration of reduced cystine sulfoxides. Their characterization is the topic of pending work.

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