lebenden Zellen in der prämitotischen Phase, wobei die zuerst ankommenden auf die später eintreffenden warten («aktive Synchronisation»). Nach Abklingen der Teilungsverzögerung treten alle Zellen zugleich in die Mitose und die folgenden Zyklusphasen ein, so dass die Strahlenempfindlichkeit der Population entsprechend dem gerade durchlaufenen Zyklusstadium schwankt. Bis die Synchronisation sich zurückgebildet hat und die Zellen wieder zufällig auf die verschiedenen Phasen verteilt sind, kann eine Reihe von Fluktuationen ablaufen. Diese spiegeln sich nach KALLMAN in periodischen Schwankungen der Strahlenempfindlichkeit von vorbestrahlten Mäusen.

Beide Modelle gehen davon aus, dass der Strahlentod beim Säugetier nach Dosen im LD50(30)-Bereich durch die Dezimierung einer einzigen Zellpopulation, nämlich der Knochenmarkstammzellen, zustande kommt und dass das Geschehen in dieser Population die Erholung der Tiere bestimmt. An bestrahlten Knochenmarkstammzellen der Maus konnte der fluktuierende Erholungsverlauf des ELKINDschen Typs auch nachgewiesen werden[22], wobei das frühe Erholungsmaximum nach 5–6 h und das darauffolgende Minimum nach 11–12 h eintrat. Die Erkenntnis, dass Vorgänge in einer einzigen lebenswichtigen Zellpopulation durch die Erholungskurven ganzer Tierkollektive widergespiegelt und dargestellt werden können, ist ein neues und wichtiges Ergebnis der Untersuchungen auf diesem Gebiet seit 1960. Diskussionen, ob die Erholung der Zellen vom subletalen Schaden oder ob ihre Synchronisation im Zellzyklus für den frühen Resistenzanstieg bestrahlter Tiere verantwortlich sind, konnten erst auf dem gemeinsamen Boden dieser Anschauung entstehen. Man wird strahlenbiologische Untersuchungen an Säugetieren deshalb künftig nicht ohne Berücksichtigung des zellulären Aspekts durchführen können[23].

Summary. A split dose experiment was performed in 12-, 24- or 32-day-old Wistar rats. About 4000 animals were used. The first dose given was 200 R whole-body X-irradiation in the 2 younger groups, and 260 R in the oldest group. At intervals from 6–48 h after the first, a second irradiation was given in order to estimate the LD50(30). No recovery was seen in terms of the LD50(30) differences between preirradiated and normal animals 6 h after the first dose. At the 12 h interval marked recovery was found in all 3 age groups, but less recovery was apparent at the later intervals.

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PRO EXPERIMENTIS

Resolution of DL-Amino Acids by Preferential Crystallization Procedure, I. Preparation of Optically Active Alanines

Although a number of optical resolutions of DL-amino acids have been reported, most of them have employed chemical or enzymatic procedures, and reports of optical resolution by physicochemical procedure have appeared less often.

If successfully applied, physicochemical resolution, especially the resolution by preferential crystallization, is considered to be one of the most advantageous procedures for the practical production of optically active amino acids[1]. However, satisfactory application of this type of simple procedure has been limited for several amino acids such as histidine[2], threonine[3] and glutamic acid[4], and it is also conceivable that there have been many failures, in spite of all efforts to apply this method to amino acids generally. So far as alanine is concerned, no report has appeared on the successful total optical resolution by preferential crystallization procedure. This communication describes a direct optical resolution of DL-alanine which has been carried out as a first approach to establish the general method for the optical resolution of amino acids.

DL-Alanine itself was proved to be unsuitable for direct resolution due to its properties of forming the racemic compound and also its solubility. Therefore, DL-alanine was converted to readily obtainable salts and derivatives, and the properties of these compounds were investigated. As a result, possibility of direct resolution was indicated in the case of alanine benzene sulphonate. Namely, the solubility of optically active alanine benzene sulphonate was much less than that of DL-modification, although its IR-spectra indicated the formation of racemic compound.

Thus conditions required for optical resolution of DL-alanine benzene sulphonate were studied in detail. As a result, pure optical isomers could be obtained from a supersaturated solution containing excess of desired antipode by seeding the solution with the crystals of the respective antipode and by filtering the precipitated crystals when the amount of the separated crystals attained was approximately twice that of the antipode initially in excess.

Alanine benzene sulphonate was prepared by dissolving DL-, D- or L-alanine in an aqueous solution of benzene sulphonic acid.

Typical resolution procedures are as follows: DL-alanine benzene sulphonate, 52.0 g, and D-alanine benzene sulphonate, 1.30 g, were dissolved in 200 ml of 97% aqueous acetone at elevated temperature and cooled slowly to 25°. The solution was seeded with 0.20 g of finely pulverized D-alanine benzene sulphonate and allowed to stand

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Experiment No.	Amount of addition		Composition of solution		Amount of inoculation (g)	Resolved crystals[a]		
	DL-form (g)	Active form (g)	DL-form (g)	Active form (g)		Yield (g)	$[\alpha]_D^{25}$	Optical purity (%)
1	26.00	(L) 0.90	26.00	(L) 0.90	(L) 0.10	2.16	+ 7.50°	100
2	2.06	0	25.74	(D) 1.16	(D) 0.10	2.68	− 7.30°	97.3
3	2.58	0	25.55	(L) 1.35	(L) 0.10	2.43	+ 7.50°	100

[a] Specific rotation of pure L-alanine benzenesulphonate: $[\alpha]_D^{25} = +7.50°$ ($c = 2$, ethanol).

at the same temperature for 16 h. The precipitated crystals were filtered and 4.40 g of D-alanine benzene sulphonate was obtained. Anal. Found: *N*, 5.62%; calcd. for $C_9H_{13}O_5NS$: *N*, 5.66%. The product was optically pure, $[\alpha]_D^{25} = -7.50°$ ($c = 2$, ethanol). The D-alanine benzene sulphonate, 3.00 g, was dissolved in 60 ml of distilled water and passed through a column of Amberlite IR-120 in H-form. The D-alanine absorbed on the resin was eluted with 180 ml of 1*N*-NH_4OH and the elute was concentrated to dryness. The residue was crystallized from aqueous methanol to give 0.98 g of pure D-alanine (91% of the theoretical). Anal. Found: *N*, 15.70: calcd. for $C_3H_7O_2N$: *N*, 15.72. $[\alpha]_D^{25} = -14.60°$ ($c = 2$, 5*N* HCl).

For further optical resolution, the mother liquor can be used repeatedly to separate the other enantiomorph. Namely, the same amount of DL-modification as that of the enantiomorph previously separated out, is added to the mother liquor and dissolved at an elevated temperature. The supersaturated solution was cooled, seeded and crystallized in the same way as described above. By repeating these procedures, L- and D-alanine benzene sulphonates were successfully obtained. The examples of the first several runs in 100 ml scale are shown in the Table.

Benzene sulphonic acid in the effluent of ion exchangers charged with solution of optically active alanine benzene sulphonate was readily recovered as DL-alanine benzene sulphonate by the addition of the corresponding amount of DL-alanine to the effluent and by further concentration of the solution.

Thus the total optical resolution of DL-alanine benzene sulphonate can be accomplished. This simple procedure is considered to be one of the most advantageous methods for optical resolution of DL-alanine, because the method requires neither optically active resolving agent nor conversion of DL-alanine into complicated derivatives.

Zusammenfassung. Die direkte optische Spaltung von DL-Alaninbenzensulfonat in die optischen Antipoden wurde bewirkt durch die bevorzugte Kristallisation aus übersättigter Lösung, die mit einem der reinen optisch-aktiven Kristalle inokuliert war.

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A Simple Accurate Method for Determining the Activity of Proteolytic Enzymes

We developed a simple modified method for the determination of free amino acids by means of ninhydrin[1], making use of a Summerson manometer and two-compartment flasks (Figure 1), widely used in conventional manometric techniques[2].

The analysis is performed as follows: 1 ml of the solution to be tested is placed in the main compartment D (Figure 1). In the corresponding sidearm C are placed 0.5 ml of 0.1% ninhydrin solution in 0.1*M* citrate buffer pH 4.7. The other compartment E contains 1 ml of 0.5*N* NaOH and the corresponding sidearm F 0.5 ml of 3*N* lactic acid. Another flask is similarly equipped, with the main compartment containing 1 ml of the citrate buffer (control flask). The 2 flasks are connected with the Summerson manometer A and allowed to thermostate in a water bath at 75 °C for 10 min and shaken at 100 cpm. The flasks are then closed by turning the three-way stopcock and the ninhydrin is added to the amino acid solution. After 10 min, the water bath temperature is lowered to 30 °C and the flasks are opened towards the exterior excluding the manometer arms, to equilibrate again the internal pressure. After 10 min the flasks are connected with the manometer arms and the lactic acid is added to the NaOH. The developed CO_2 quantities are calculated at the end of the variations of manometric pressure. The CO_2 coming from the decarboxylation of the free or terminal amino acid is calculated by subtracting, from the amount of CO_2 developed in the flask containing the amino acid, the amount of CO_2 developed in the flask without amino acid.

The method is useful for substances developing 1 to 10 μmoles of CO_2. The method gives accurate results even using non-CO_2-free reagents, as it results from Table I.

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