washed and centrifuged 5 times, dried *in vacuo*.

The measurements reported in this paper were made without cover quartz plates. The work with cover plates is easier, it does not require as much of care in keeping temperature and humidity constant; but the cover plates reduce the relative remittance of the sample.

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Specific Radioactivities of Protein Discs in a Polyacrylamide Gel

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Turn-over rates of proteins are heterogeneous and, therefore, determination of a turn-over rate from a mixture of proteins yields only little information concerning a single species. Application of polyacrylamide gel electrophoresis has emerged as a method of choice for simultaneous measurement of specific radioactivities of an array of purified proteins.

The procedure introduced in this paper is highly reproducible allowing determination of absolute specific radioactivities of individual discs in a polyacrylamide gel. The stained protein bands are cut from a gel on a device producing cylinders of equal size, followed by solubilization and protein estimation by means of the bound dye. The solutions are then evaporated to dryness and processed for liquid scintillation counting.

Experimental. Samples: Rat brain cortex was used in all experiments. For the incorporation study 20 μCi(^{3}H)-leucine (20 μl) was injected into a lateral ventricle and the animals were killed at various times. The cortices were homogenized and centrifuged at 100 000 *g* for 30 min to yield the soluble proteins in the supernatant and the insoluble proteins in the pellet; both fractions were then treated with "the sample solvent"[1] for electrophoresis. The solvent contained 50 mM K_2CO_3, 8 M urea, 10 % mercaptoethanol, and 5 % Triton X-100.

Electrophoresis. Polyacrylamide gel electrophoresis was performed according to Lim *et al.*[1] by replacing bis(*N*,*N'*-methylene bisacrylamide) by 0.4 % ethylene diacrylate (Borden Chemical Co.) in the small pore gel solution to make the gel alkali labile.[2] 200 μl of the sample was applied onto 30 × 0.5 cm columns and a current of 3 mA/tube was connected until the methyl green marker had migrated to the cathode end of the gel.

Quantitation of protein and radioactivity. The gels were stained with 0.5 % amido black in 10 % acetic acid for 30 min; the destaining was made by diffusion in a solution containing 10 % acetic acid and 20 % ethanol.

A sketch was drawn to indicate the positions of the major bands. All parallel gels were placed on a slicer (Fig. 1) adjusted to produce 2 mm cylinders and the major bands were cut according to the sketch making sure that each band was contained in a cylinder. To provide background readings for protein and radioactivity four additional slices were taken from such parts of the gel which were apparently devoid of protein. The slices obtained were dissolved in 1 ml 1 M NH_4OH and the absorbance of the solution was measured at 625 nm[3] followed by evaporation to dryness at 80°. The residue was mixed with 200 μl of Soluene 100 (Packard), 20 μl of water and 10 ml of toluene scintillation fluid and counted in a Packard scintillation spectrometer to a small counting error. The results were corrected for color quenching by internal standardization. The quenching was at most 10 % of the total counts. The counting efficiency was 15 %.

Standard curve for protein. To relate the amido black absorbance and protein content, protein discs were dissolved in NH_4OH and the absorbance was measured at 625 and 750 nm. The samples were then evaporated and dissolved in NaOH followed by estimation of protein content by Lowry's method[4] at 750 nm. The reading for amido black at 750 nm was subtracted from that obtained with Lowry's method.

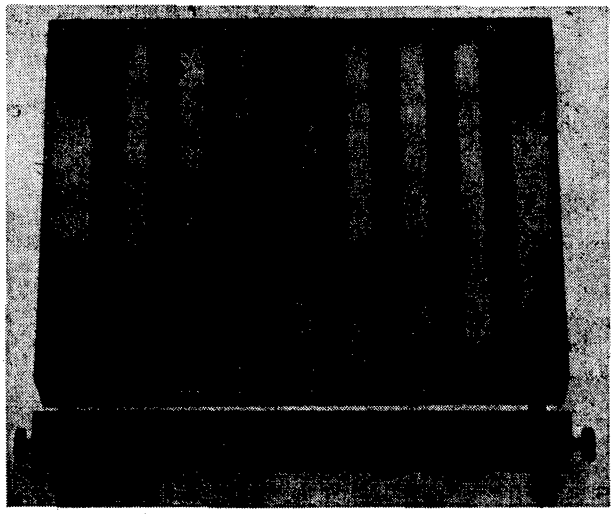

Fig. 1. The device used for cutting gels.

Results and discussion. Validity of amido black for protein estimation. The relationship between amido black and 12 different soluble and insoluble proteins is tested in Fig. 2. The amido black absorbance appears to relate very well to the soluble protein; while with the insoluble protein maximally a 17 % deviation from the linearity is noted. This may, however, well be an erroneous reading of Lowry's method produced by a purified protein sample.[4] We conclude that when the same protein discs are compared in different gels, amido black can be used as a sensitive and reliable

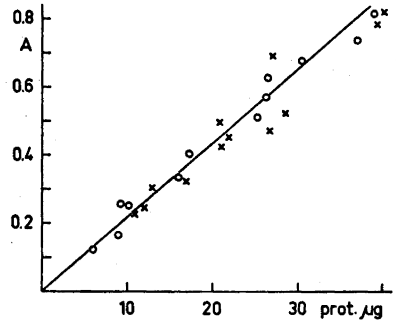

Fig. 2. Standard curve relating amido black absorbance and protein content. 12 different soluble (O) and insoluble (×) proteins were analyzed.

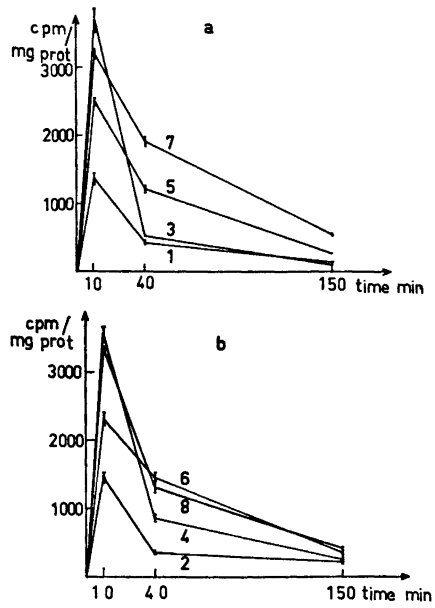

Fig. 3a and b. Incorporation of radioactivity into brain insoluble proteins 10, 40, and 150 min after intraventricular injection of 20 µCi ^{3}H-leucine. Proteins were separated by polyacrylamide gel electrophoresis and specific activities of 8 different bands were determined. Band No. 8 is closest to the origin. Standard errors indicated (No 3–5).

protein binding dye. Coomassie blue and procion blue, suggested by some authors [5] for protein staining, are not suitable in this procedure, because they are alkali labile. To review some other methods used for protein estimation in gels, densitometry [6] is only semiquantitative, and the use of continuously labelled proteins [7] is expensive and in many *in vitro* experiments impossible.

Reproducibility of the procedure. In Fig. 3 a and b rat brain insoluble proteins are analyzed 10, 40, and 150 min after the injection of ^{3}H-leucine into a lateral ventricle. In all cases the standard errors are smaller than 10 % (N = 3 – 5). Similar techniques introduced by other workers [3,8–11] have, in our opinion several shortcomings, and the authors did not demonstrate the reliability of their results by statistical treatment.

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A Ti_3O_5 Modification of V_3O_5-type Structure

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This note will report briefly on a new modification of Ti_3O_5, studied at the above Institutes.

One of us (G. Å.) has made systematic preparative studies by heating appropriate amounts of TiO_2 and Ti_2O_3 (both about 99.97 % pure) in sealed, evacuated silica tubes at different temperatures for varying periods of time. The tubes were quenched in water and the preparations characterized by their X-ray patterns taken at room temperature.

A phase giving a powder pattern previously not encountered for Ti_3O_5 was found in samples heated at temperatures within the range 600 – 925°C. Single-phase specimens were obtained at high temperatures, 900 – 925°C, after long heating (more than a month). Samples held at higher temperatures gave the pattern of a Ti_3O_5 modification (β-Ti_3O_5) previously shown by two of us to be formed by a rapid transformation from a high-temperature modification of Ti_3O_5 of pseudo-brookite structure (α-Ti_3O_5).[1] The latter phase is evidently the one which forms at temperatures exceeding about 950°C.

It was possible to index the powder pattern of the new phase (γ-Ti_3O_5) from its similarity to that of V_3O_5 (*cf.* Table 1). The following dimensions were derived for the unit cell chosen to conform with the orientation previously used for V_3O_5.[2,3]

The data given here for V_3O_5 were obtained by S. Å., who also performed a refinement of the structure of this substance. An account of the results, based on 633 independent reflections (diffractometer data) and carried to a conventional R value of 2.9 %, will shortly appear elsewhere.

Independent preparative and X-ray studies conducted by H. O. have given results in agreement with those described above. It was found that α-Ti_3O_5 once formed did not transform into γ-Ti_3O_5 even