R-factor had not been introduced, was incubated under the same conditions with the irradiated recipient. Since the donor strain is unable to utilize thymine and the chromosomal DNA of the recipient is unable to replicate, the incorporation excess is ascribed to the synthesis of the DNA of the R-factor after transfer to recipient.

Although incorporation of [^3H]thymine during incubation of the irradiated recipient strain with the donor strain lacking the R-factor was less than that observed during transfer of the R-factor, some incorporation did occur. In contrast, much less incorporation occurred when the two strains were incubated separately with [^3H]thymine. It was found that incubation of the irradiated recipient strain with [^3H]thymine gave rise to [^3H]dTMP in the medium. Moreover, this material, when added to a culture of the donor strain, was incorporated efficiently in trichloroacetic acid-insoluble material. It is believed that the conversion of thymine into dTMP followed by incorporation of this compound into the DNA of the donor bacterial accounts for the incorporation of radioactivity into DNA in these experiments in the absence of transfer of R-factor.

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Genetic Expression of Sex-Factor after Transfer into Irradiated Cells of *Escherichia coli* *uvrA*⁻

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It has been demonstrated that, after transfer of the episome into cells of *Escherichia coli* previously killed by u.v. irradiation, synthesis of DNA of the F-merogenote (Freifelder & Freifelder, 1968) and of the R-factor Rldrd19 (Silver & Falkow, 1970; Barker, Hardman & Twose, 1971) takes place even though chromosomal DNA synthesis is suppressed in the recipient cells. Experiments have shown (N. Hardman & T. M. Twose, unpublished work) that cells subjected to doses of approx. 7000–8000 erg/mm² are non-viable and exhibit no measurable synthesis of protein or increase in cell mass. The present experiments are concerned with the ability of irradiated cells to support the normal functions of sex-factors.

The F'-lac episome was transferred to a suitable mutant of *E. coli*, which was then subjected to appropriate conditions for induction of the lac operon. It was found that induction of β -galactosidase activity was completely blocked by u.v. irradiation of the recipient with a dose of 7400 erg/mm² before introduction of the episome. At lower doses partial inhibition of the induction was observed. Analogous experiments to investigate the possible formation of enzymes following transfer of R-factor present special difficulties and have not yet been carried out.

The presence of sex-factor in a viable cell confers the ability to act as donor in conjugation with a second recipient. U.v. irradiation of recipient cells with a dose of 7400 erg/mm² before acceptance of either of the episomes Rldrd19 and F'-lac completely prevented the ability subsequently to act as donor in conjugation. With lower doses of irradiation partial inhibition was observed. It is concluded that the episomes do enter the u.v.-irradiated cells, since synthesis of DNA takes place after incubation of mixed cultures under conditions suitable for conjugation (Barker *et al.* 1971), but that expression of episomal genes probably does not take place.

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Protein Content of Myelin

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Determination of the protein content of membrane preparations is difficult because of the large amounts of lipids not readily dissociable from the proteins. Systematic study of the problem has been made by several methods to find a valid procedure for comparison of protein contents of central-nervous-system myelins from different animal species and of membranes from different tissues.

Removal of lipids by a variety of solvents from freeze-dried rat central-nervous-system myelin and gravimetric measurement of the protein residue yields results dependent on solvent (Winder & Gent, 1970a), but suggests that protein does not exceed 20% (w/w).

By taking the nitrogen content of myelin to be

$3.8 \pm 0.2\%$ (five determinations) together with rat myelin lipid composition (Cuzner, Davison & Gregson, 1965) for which there is $12.9 \mu\text{g}$ of N/mg of myelin lipid, and an assumed protein nitrogen content of 16%, then the protein content of rat myelin is 17%. Similar treatment of ox and human myelin nitrogen contents (Wolfgram & Kotorii, 1968) leads to values of 19% protein.

The difference between extinction values at 215 and 225 nm is proportional to protein concentration in solution (Waddell, 1956) and depends on peptide-bond absorptions. Because of light-scattering by particulate myelin suspensions anomalous protein contents are to be expected (Winder & Gent, 1970b), differing from instrument to instrument because of the variable inclusion of forward-scatter components in the transmitted beam. After appropriate corrections rat myelin protein averages at 44%. Part of the myelin absorption in the short-u.v. region is shown to be due to cerebroside, and allowance for this effect lowers the protein content to 28%. Solubilization of myelin by lysophosphatidylcholine by the procedure of Gent, Gregson, Gammack & Raper, (1964) eliminates turbidity, and with correction for cerebroside absorption a protein content of 20% is indicated. Lipid-protein interaction may modify the chromophore function of peptide linkages. Results are presented that indicate considerable modification by lipid interactions of the chromophores responsible for 280 nm absorption.

Estimates by the tyrosine-dependent method of Lowry, Rosebrough, Farr & Randall (1951) suggest for rat myelin a protein content of 20.0%, S.D. 1.3% (17 determinations). Results are also reported for other species and different brain regions by this method.

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Improved Separation of Viable Neurons and Glia from Rat Brain

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The preparation of greatly enriched fractions of neurons and glial cells in workable amounts is requisite for a detailed study of glia-neuron synergic relationship at the biochemical level. Information derived from the application of the method of Rose (1967) is subject to some doubt on the grounds of reliability of the fractionation technique and integrity of the separated cells (Cremer, Johnston, Roots & Trevor, 1968). Norton & Poduslo (1970) introduced considerable technical improvement by using trypsin before tissue disruption. With a modified disruption procedure and zonal centrifugation their method has now been extended to yield large numbers of viable cells.

Seven brains of 14–40-day-old rats were cut into small pieces ($0.5\text{--}1.0 \text{ mm}^3$) and incubated for 60 min at 37°C with 10 vol. of trypsin medium (5% fructose–5% glucose–1% serum albumin–1% Difco 1:250 trypsin–100 mM-sodium-potassium phosphate buffer, pH 6.2). After three rinses in cold trypsin-free medium the pieces of tissue were disrupted on 110-mesh bolting cloth stretched over a glass funnel by gentle abrasion with a large paint brush, while the medium was continuously dropped on the preparation. The suspension was filtered three times through stainless-steel sieves (150 and 200 mesh). In an attempt to separate cells from processes, the sample (15–20 ml) was loaded on to a discontinuous sucrose gradient comprising a lower layer of 60% sucrose (10 ml) with superjacent 22% sucrose prepared in the medium (10 ml). After 5 min centrifugation at 3000 rev./min in an MSE Mistral 6L centrifuge, cells were collected from the 22%/60% sucrose interface and loaded on to a continuous sucrose gradient (40–60% in medium) in an MSE H.S. zonal rotor. After centrifugation for 30 min at 4500 rev./min 7.5 ml or 15 ml samples were collected.

The cells were identified under phase-contrast illumination and estimated as percentage of total brain DNA by the diphenylamine method (Burton, 1956). They comprised six different cell fractions located as follows: glia A (40% sucrose, 3% DNA); glia B (42% sucrose, 2% DNA); capillaries (46% sucrose, 2% DNA); neurons and capillaries (49% sucrose, 5% DNA); neurons A (53% sucrose, 8% DNA); neurons B (58% sucrose, 4% DNA). The characters of the cell types will be described.

Viability was tested by measuring the incorporation rate of DL-[^{14}C]valine *in vitro*. When referred to DNA, the incorporation rate of glial cells was