relatively small length of the PL1 molecule eliminate the possibility of cross-linkage of small free DNA zones in dNP by preparations PL1 and PL2. Thus 38% of the 'free' DNA zones are sufficiently long to accommodate either preparation PL1 or PL2. With preparation PL3, cross-linkage probably does occur and may account for its slightly higher titration value. The even higher values for preparation PL1 and for Toluidine Blue are presumably related to their smaller size.

Previous enzyme studies (R. F. Itzhaki, unpublished work) had shown that there are few, if any, extensive truly free DNA zones in dNP. This and the present result can be explained on the basis that dNP contains zones of phosphate groups unbound but hidden beneath protein. The distribution of basic residues in histones is very irregular (DeLange, Farnbrough, Smith & Bonner, 1969), and model-building studies (Richards & Pardon, 1970) with the F2a1 histone suggest that less than 60% of the DNA phosphate groups in dNP can be neutralized by the basic residues. The remaining phosphate groups would not be estimated as free by the enzyme method because of their associated protein, but would be free to bind dyes and polylysine. The relevance of the results to the role of histones in control of gene transcription will be discussed.

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Investigations into the Nucleotide Sequence of Cysteine Transfer Ribonucleic Acid from Baker's Yeast

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Cysteine tRNA has been isolated from baker's yeast RNA by a modification of the reversed-phase partition-chromatographic system described by Kelmers, Novelli & Stulberg (1965).

Cysteine tRNA represents about 1% of the RNA applied to the column and after chromatography is enriched at least 20-fold in its ability to accept [¹⁴C]cysteine in the standard assay system with a partially purified aminoacyl-tRNA synthetase prepared from rat liver. In our hands the maximum incorporation of [¹⁴C]cysteine corresponded to about 40% of that calculated, assuming mol.wt. 25000 for cysteine tRNA.

Our preparation does not accept significant quantities of serine, phenylalanine, tyrosine, iso-

leucine, proline and alanine. Further, there is no incorporation of radioactivity from a mixture of all ¹⁴C-labelled amino acids lacking cysteine. It is not further resolved by chromatography in other systems nor by countercurrent distribution for 500 transfers in a phosphate-formamide-propan-2-ol system (Apgar, Holley & Merrill, 1962). The elution patterns resulting from enzymic degradation described below are consistent with the view that this RNA preparation is substantially pure.

The nucleotide composition was established by DEAE-cellulose chromatography of an alkaline or ribonuclease T2 digest, with 0.07M-sodium acetate buffer, pH 4.10 or 4.15, as eluent. Oligonucleotides resulting from complete pancreatic and T1 ribonuclease hydrolyses have been isolated and partially characterized. Minor nucleotides identified as constituents of these oligonucleotides include ψ p, Tp, 5-Me-Cp, diMe-Gp and a nucleotide resembling isopentenyl-Ap.

Treatment with ribonuclease T1 at 2°C for 45 min affords two fragments that can be separated by DEAE-cellulose chromatography in 7M-urea at pH 7.9. With a higher enzyme:RNA ratio a more complex series of products is obtained. The sequences of these larger oligonucleotides are still to be determined.

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Chemotaxonomy and Biosynthetic Relationships of Boletineae Pigments

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Morphological taxonomy of the Boletineae is attended with the disadvantage of subjective assessment of essentially qualitative features. The nature of the pigments and the presence or absence of a polyphenol oxidase in eight species of three Boletineae genera have therefore been examined as a possible aid in taxonomy.

T.l.c. of fungal extracts separates polyhydroxy-diphenylbenzoquinones and pulvinic acid derivatives, which have been identified by spectroscopic methods and colour reactions. Some *Boletus* species turn blue on bruising and this has been attributed to enzymic oxidation of variegatic and xerocommic acids, which are pulvinic acid derivatives (Steglich, Furtner & Prox. 1968; Beaumont, Edwards & Elsworth, 1968). In a study of eight Boletineae species four pulvinic acids have been isolated.

These were variegatic acid and xerocomic acid, and two new pulvinic acids, only one of which exhibited the blueing reaction. Of taxonomic value is the presence or absence of the polyphenol oxidase responsible for the blueing. In this respect, a species of the *Boletus bicolor* group did not turn blue although variegatic acid was present. Extracts of two *Suillus* species contained four pigments, which are considered to be polyhydroxydiphenylbenzoquinones on the basis of their spectral properties and colour reactions.

The genus *Paxillus* has morphological affinities with both Boletineae and Agaricineae. A blueing *Paxillus* species afforded variegatic acid and a new non-blueing pulvinic acid. *Paxillus atrotomentosus* elaborates a tetrahydroxydiphenylbenzoquinone, atromentin (Kögl & Postowsky, 1925). The co-occurrence of the benzoquinone and pulvinic acids in *Paxillus* suggests that this is a chemotaxonomic link between the sub-orders.

The diphenylbenzoquinones are readily oxidized to pulvinic acids and the biological oxidation is probably catalysed by a pyrocatechase-type enzyme. Pyrocatechase oxidizes catechol to *cis-cis*-muconic acid (Knox, 1961). A biosynthetic scheme accounting for the production of blueing and non-blueing pulvinic acids will be presented.

Paxillus involutus elaborates involutin, a polyhydroxydiphenylcyclopentenone (Edwards, Elsworthy & Kale, 1967). The biosynthesis of this metabolite may be explained in terms of a by-pass from the pulvinic acid pathway and appears to involve a decarboxylation of an intermediate muconic acid derivative, cyclo-dehydration and reduction.

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The Use of Zonal Centrifugation in the Preparation of Glomeruli and Tubular Fragments from Rat Kidney

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A number of methods have been used for the isolation of glomeruli and of tubular fragments from a variety of animals. Greenspon & Krakower (1950) separated glomeruli from a cortical mash of

dog kidney by means of a sieve of known mesh size. Fong & Drummond (1968) used a similar procedure for rat kidney, and van Breda Vriesman & Willighagen (1968) digested dog kidney with collagenase before sieving. Collagenase has also been used in the preparation of rabbit renal tubules (Burg & Orloff, 1962). These preparations have been used in the production of antisera, in the preparation of basement membranes and in studies on active transport. Mattenheimer, Pollak & Muehrcke (1970) isolated different regions of the human nephron by micro-dissection and compared their enzyme content. The development of a simplified procedure for the simultaneous preparation of large quantities of glomeruli and tubular fragments would allow a detailed study of the enzymic content and isoenzyme forms present in normal and experimentally damaged kidneys.

Male Sprague-Dawley rats (200 g) were killed by a blow on the head, and the renal arteries and veins were exposed and clamped. Then 5 ml of a 0.05% collagenase solution in 0.9% NaCl (adjusted to pH 8.5 with 0.25 M-NaOH) was injected into each renal pelvis. After 10 min the kidneys were dissected out, the renal capsules removed and the cortices separated from the medullae. A known weight (4–16 g) of cortical preparation was then minced to a paste with a razor blade and made up to a 5% suspension in the collagenase solution. The suspension was forced through a no. 1 Luer needle and incubated at 25°C with constant stirring. After 1 h the suspension was filtered through two layers of muslin and the filtrate was washed three times by centrifugation at 5000 g-min on an MSE 18 ultracentrifuge and suspension of the pellet each time in 100 ml of 0.9% NaCl (pH 7.2). The final pellet was suspended in 50 ml of 40% (w/w) sucrose solution (pH 7.2).

The suspension was fractionated by zonal centrifugation in an MSE type A zonal rotor at 5000 rev./min for 45 min at 4°C. A continuous density gradient of 40–60% (w/w) sucrose was used and fractions (35 ml) were collected. Four bands were apparent in the rotor and the fractions corresponding to them were characterized by light-microscopy. A sharp band at 50–51% (w/w) sucrose contained decapsulated glomeruli and was distinct from a larger band, containing tubular fragments, at 45–48% (w/w), and both were well separated from erythrocytes and cell debris. Glomeruli and tubular fragments were recovered from these fractions by centrifuging in an MSE 18 ultracentrifuge at 500 000 g-min, and the resulting pellets were suspended in a small volume of 0.45 M-sucrose solution.

Acid and alkaline phosphatase, leucyl- β -naphthylamidase and *N*-acetyl- β -glucosaminidase activities were assayed in the two preparations and their