method the GG are precipitated in the top layers of a cellulose column saturated with 1% aqueous cetylpyridinium chloride. The GG are then eluted from the column by salt solutions of increasing concentrations, and the amount in each fraction is determined by the Elson and Morgan method.

Tests with pure GG as well as with various connective tissues show a quantitative recovery. Several experiments carried out on a homogenized dry powder of horse nasal cartilage show that a reproducible elution pattern is obtained. The method has been applied to serial sections cut through the various layers of the nasal septum, with alternate sections for histological control. Various layers showed characteristic GG pattern. Corresponding patterns were also obtained in these histologically distinct structures when separated by microdissection of sections. Similar results were obtained also with other connective tissues.

The procedure gives reproducible results and a characteristic pattern for the tissue structures examined. Even if the GG fractions have not yet been fully characterized, the constancy of the pattern indicates that they represent real entities and not artefacts produced during extraction and fractionation.

Antonopoulos, C. A., Borelius, E., Gardell, S., Hamnström, B. & Scott, J. E. (1961). *Biochim. biophys. Acta*, **54**, 213.
Scott, J. E. (1955). *Biochim. biophys. Acta*, **18**, 428.
Scott, J. E. (1960). In *Methods of Biochemical Analysis*. Ed. Glick, D., **8**, 145. New York: Interscience Publishers.

The Metabolism of Ubichromenol in the Rat

By D. L. Laidman and R. A. Morton. (*Department of Biochemistry, University of Liverpool*)

The chemical relationship between the ubiquinones and ubichromenols (Laidman, Morton, Paterson & Pennock, 1960; Isler *et al.* 1960) raises the question of a biochemical relationship, e.g. the two compounds might play a part in oxidative phosphorylation (Lehninger, 1960). Attempts have therefore been made to detect any *in vivo* conversion of a foreign ubichromenol to the corresponding ubiquinone in the rat. Ubiquinone (45) was accepted as the natural isoprenologue for the species.

Samples of pure ubichromenol (30) and ubichromenol (50) were prepared from pure samples of the corresponding ubiquinones according to the method of Links (1960) except that acid-washed Brockmann grade 2 alumina was used in place of Links's neutral Brockmann grade 0 alumina. Infrared spectra and quantitative ultraviolet spectra confirmed that good preparations had been obtained.

Each rat in a group of adults was injected intramuscularly with 1·0 mg. ubichromenol (50) in 0·1 ml. arachis oil and each rat in a control group was injected with 0·1 ml. arachis oil only. After 48 hr. the rats were killed and their liver, kidneys, intestines and injected muscles removed and appropriately bulked for analysis. About 20% of the injected ubichromenol (50) remained at the site of injection but 80% could not be accounted for either as ubiquinone or ubichromenol in any of the tissues examined. Intramuscular injection of ubichromenol (30) into weanling rats gave essentially the same result in that conversion of the injected ubichromenol (30) to ubiquinone (30) could not be detected.

Rat urine collected for 48 hr. after the intramuscular injection of urichromenol (50) in arachis oil or Tween-20 showed no detectable ubiquinone or ubichromenol but contained purple pigments solubilized as the colourless glucuronide or ethereal sulphate. The pigments could be released from their complexes by acidification and could then be extracted into ether. Similar pigments were excreted in the urine after the injection of ubichromenol (30) and ubiquinone (50).

The pigments showed $\lambda_{max.}$ 285 mμ ($E^{1\%}_{1\,cm.}$ 150) and 505–515 mμ ($E^{1\%}_{1\,cm.}$ 34) in ethanol and the action of borohydride suggested either a 1,2-benzoquinone or a hydroxy-1,4-benzoquinone (cf. tocopurple: Frampton, Skinner, Cambour & Bailey, 1960). The insolubility of the pigment in cyclohexane points away from an aliphatic side-chain like those of ubiquinone and ubichromenol. The origin of these pigments is unknown.

Frampton, V. L., Skinner, W. A., Cambour, P. & Bailey, P. S. (1960). *J. Amer. chem. Soc.* **82**, 4632.
Isler, O., Rüegg, R., Langemann, A., Schudel, P., Ryser, G. & Würsch, J. (1960). *Ciba Found. Symp. 'Quinones in Electron Transport'*, p. 79.
Laidman, D. L., Morton, R. A., Paterson, J. Y. F. & Pennock, J. F. (1960). *Biochem. J.* **74**, 541.
Lehninger, A. L. (1960). *Fed. Proc.* **19**, 592.
Links, J. (1960). *Biochim. biophys. Acta*, **38**, 193.

Chromatography of Denatured Deoxyribonucleic Acid on Calcium Phosphate

By G. Bernardi. (*Centre de Recherches sur les Macromolécules, Strasbourg, France*)

The chromatographic behaviour of native deoxyribonucleic acid (DNA) on calcium phos-

phate has been described (Bernardi, 1961). Further work showed that the elution curve obtained with heat-denatured DNA (from chicken erythrocytes or calf thymus) was remarkably different. Essentially the same elution schedule with phosphate buffers of pH 6·8 and increasing molarities was used as previously, with the difference that all eluants and the starting DNA solution contained 1% (w/v) formaldehyde. Under these conditions native DNA was mainly eluted at 0·25M-phosphate buffer, with a smaller fraction at 0·20M and a minor one at 0·50M. When heated at 100° for 10 min. in their formaldehyde-containing buffers and then cooled rapidly, all fractions showed the same hyperchromicity of about 38% at 260 mμ as the starting DNA.

DNA which had been heated at 100° for 15 min. in 0·13M-NaCl–0·01M-phosphate buffer, pH 6·8, and then cooled rapidly (Doty, Marmur, Eigner & Schildkraut, 1960) was mainly eluted at 0·15M-phosphate buffer with smaller fractions at 0·10M, 0·20M, 0·25M, and a minor one at 0·50M. As in the case of native DNA, elution was quantitative. Whereas the starting denatured DNA showed a hyperchromicity of less than 5% upon heating in the presence of formaldehyde, the 0·10M and 0·15M fractions showed no hyperchromic effect, and the 0·25M and 0·50M fractions showed a hyperchromicity of about 15%. After two more chromatographic runs, the 0·25M fraction did not change significantly in its hyperchromicity. Also it was shown that a separation of an artificial mixture of native and denatured DNA was possible to a very large extent, in only one chromatographic run. This eliminates the possibility of the presence of a mixture of native and denatured DNA in the fraction and suggests the existence of partially denatured molecules.

More recent work showed that it was possible to change at will the proportions of the various fractions just by changing the temperature of denaturation. As it is known (Sueoka, Marmur & Doty, 1959) that the denaturation temperature is a function of the average base composition of DNA, one might expect a difference of composition in the different fractions. Preliminary results indicate that this is the case.

Bernardi, G. (1961). *Biochem. biophys. Res. Commun.* **6**, 54.
Doty, P., Marmur, J., Eigner, J. & Schildkraut, C. L. (1960). *Proc. nat. Acad. Sci., Wash.*, **46**, 461.
Sueoka, N., Marmur, J. & Doty, P. (1959). *Nature, Lond.*, **183**, 1429.

Fractionation of Nucleic Acid-like Material in Extracts of Rat Spleens

By J. D. Hawkins. (*Department of Biochemistry, Medical College of St Bartholomew's Hospital, London, E.C. 1*)

In searching for a way of separating protein fractions from extracts of rats' spleens following injection of heterologous proteins, chromatography on DEAE-cellulose has revealed a number of fractions believed to contain nucleic acids. This belief is based on their ultra-violet absorption spectra and the fact that they contain ^{32}P after injection of $^{32}PO_4^{3-}$ into the rats. As these substances are extracted with dilute salt solutions they are probably ribonucleic acids.

Spleens were homogenized with 0·10M-NaCl–0·05M-sodium citrate (Hawkins & Haurowitz, 1961) and the red-brown extracts dialysed against several changes of 0·02M-sodium citrate. The small precipitate which separated was discarded, and the supernatant applied to a column of DEAE-cellulose 5 cm. high × 2·5 cm. diameter. This was eluted successively with 0·02M-sodium citrate, 0·20M-NaCl, 0·50M-NaCl, 1·00M-NaCl, 0·10N-NaOH–0·90M-NaCl, and 1·00N-NaOH. Recovery of material absorbing at 260 mμ and 280 mμ and of ^{32}P was at least 85%. About a third of such material (including all the coloured material) was apparently not retained by the column as it was eluted by 0·02M-sodium citrate. The fraction eluted by 0·20M-NaCl appeared to contain protein and very little nucleic acid as the ratio of absorbencies at 280 mμ/260 mμ was greater than 1·0. Material eluted by higher concentrations of NaCl and by NaOH appeared to contain nucleic acids as the ratio of absorbency at 260 mμ/280 mμ was greater than 1·0 and was characteristic for each fraction.

When rats were injected with $^{32}PO_4$ and killed at intervals up to 4 days later, ^{32}P was observed to enter these nucleic acid fractions slowly. After 2 hr. only about 0·01% of the injected ^{32}P was incorporated into the dialysed extract and maximal incorporation (0·04% of the injected dose) was observed after 47 hr. Only very small amounts of ^{32}P were found in the first two fractions eluted. Up to 47 hr. after injection the fraction eluted by 0·10N-NaOH–0·90M-NaCl contained the greatest amount of ^{32}P, but by 96 hr. after injection the greatest amount of ^{32}P was eluted by 1·00M-NaCl. At all times this fraction had the highest specific activity (counts/min./unit of absorbency at 260 mμ) of all the fractions.

The material eluted by 1·00N-NaOH presumably contained degraded nucleic acids since it was shown to diffuse freely through a dialysis membrane.