PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **324th Meeting** *of the Biochemical Society was held in the Department of Biochemistry, The London Hospital Medical College, Turner Street, London, E.* 1, *on Saturday,* 12 *December* 1953, *at* 11 *a.m., when the following papers were read:*

COMMUNICATIONS

Diffusible Peptide-like and Glycosidic Constituents of Italian Rye Grass. By R. L. M. Synge and J. C. Wood. (*Rowett Research Institute, Bucksburn, Aberdeenshire*)

Data collated by Pardee (1951) suggest that phenol should be a useful selective solvent for extracting peptides. In attempts to purify the diffusible neutral 'bound amino acid' of Italian rye grass (fraction 3-*M*, Synge, 1951; N, approx. 0·1 % of dry matter of fraction) a 5-unit counter-current distribution with phenol-water yielded most of the nitrogenous material in the phenol-rich extracts while eliminating higher saccharides and most of the monosaccharide present. Doing counter-current distribution before electrophoretic fractionation increased the amount of material conveniently handled.

Besides peptide-like material, this procedure concentrated water-soluble glycosidic material. Acid hydrolysis (N-HCl, 2 hr. at 100°) liberated water-insoluble material (partly soluble in ether, entirely in ethanol) and glucose (with smaller amounts of galactose and rhamnose). A preparation from spring grass gave: reducing sugar liberated (as glucose), 0·41 %; ether-extractable and water-insoluble material, 0·64 % of dry matter of original grass. Corresponding figures for a late flowering stage were 0·10 and 0·20 %, fluorescent and pigmented materials being likewise present in smaller amounts. These glycosides may be lignin precursors; Manskaya & Bardinskaya (1952) noted similar changes during lignification.

Preparations were further fractionated by partition chromatography (phenol-water; phenol phase stationary) on dichlorodimethylsilane-treated kieselguhr (Howard & Martin, 1950). The peptide-like material (detected according to Rydon & Smith (1952), by N determination, etc.) emerged from the chromatogram without well-defined peaks. However, rechromatography of any cut gave a peak in the corresponding position, demonstrating effective fractionation of heterogeneous material. Acid hydrolysis (as above) broke few peptide bonds and permitted removal of the aglycone fraction. On chromatography reducing sugar was then concentrated in early effluent fractions, and peptide material, whose chromatographic behaviour was not much affected by the acid treatment, was obtained less contaminated in later fractions (N, 1–6 % of dry matter). Cuts from phenol-water chromatograms have been further fractionated on kieselguhr chromatograms (*n*-butanol-acetic acid-water systems); some cuts also give well-defined fractions on paper chromatography with water or aqueous KCl. A complicated series of compounds is thus revealed.

Phenol likewise selectively extracts the acidic peptide-like material of fraction 4-A (Synge, 1951). On further fractionation by paper electrophoresis, the Rydon test revealed a complicated picture (cf. Turba & Esser, 1953). We are further exploring the use of phenol for extracting peptide material from indiffusible grass fractions.

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Porphyrins in the Urine of Normal Men. By A. Comfort and M. Weatherall. (*Department of Pharmacology, London Hospital Medical College, London, E.* 1)

The porphyrins present in the urine of healthy young adult males have been extracted by adsorption at pH 2–2·5 on talc, esterified with methanolic sulphuric acid and chromatographed from benzene with increasing concentrations of chloroform and finally with chloroform containing methanol on alumina columns. The fractions were examined by paper chromatography with lutidine-water after acid hydrolysis (Nicholas & Rimington, 1951). Seven batches, of 2·5–29 l., were examined. All contained predominantly coproporphyrin. All but one yielded at least two other porphyrin bands on

the alumina column. These bands were less easily eluted than coproporphyrin and on paper chromatography gave mixtures of porphyrins with up to five constituents all with R_F values lower than that of coproporphyrin (mean 0·52, 0·43, 0·33, 0·13 and 0·07; coproporphyrin = 0·67, uroporphyrin = 0·07 on these papers). The properties of these fractions appear similar to some of those recently reported in much larger amounts in porphyric urines.

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Intramanometric Dialysis. By H. Laser. (*Molteno Institute, University of Cambridge*)

The oxidation of ethanol by catalase has so far only been obtained in presence of an enzymic system, like amino acid oxidase, xanthine oxidase or notatin + their specific substrates, the oxidation of which provides a continuous supply of hydrogen peroxide (Keilin & Hartree, 1936, 1945). When H_2O_2 as such is added to catalase + ethanol, only an insignificant fraction of it is used in peroxidatic oxidation, the main portion being catalytically decomposed to H_2O and O_2.

In order to obtain a greater efficiency in peroxidatic oxidation of ethanol by catalase on adding H_2O_2 as such, the following conditions had to be fulfilled: H_2O_2 had to be introduced (1) continuously for a certain period of time, (2) at a predetermined measurable rate, (3) in variable concentrations. This, it was thought, could be obtained by admitting the peroxide by dialysis.

A manometer vessel has therefore been devised which fulfils these conditions. It consists of an upper and a lower part which are divided by a cellophan membrane. The upper part contains the enzyme while the lower part is filled with a buffer solution. This part is provided with a Dixon-Keilin tap of the type introduced by these authors in their method for the determination of the respiratory quotient (Dixon & Keilin, 1933). Its cavity contains the substance to be dialysed, e.g. H_2O_2. On turning the tap 'on' so as to connect it with the lower part of the vessel H_2O_2 is released into the buffer solution and dialysis into the upper part of the vessel commences. In this way the rate of diffusion of H_2O_2 has been measured manometrically by the rate of liberation of O_2 by catalase. The rate of diffusion, as was to be expected, decreases exponentially and is proportional to the H_2O_2 concentration. The rate of decomposition of H_2O_2 was within wide limits independent of the catalase concentration. The application of intramanometric dialysis for the study of other biological problems will be briefly discussed.

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Peroxidatic Activity of Catalase. By H. Laser. (*Molteno Institute, University of Cambridge*)

The peroxidatic activity of catalase has been studied by means of intramanometric dialysis (see previous communication), whereby H_2O_2 of known concentration is introduced at a predetermined rate into a buffer solution containing ethanol and catalase. If peroxidatic oxidation of ethanol to aldehyde takes place, less O_2 is liberated from peroxide than in the control in absence of ethanol. The greater the difference the more the aldehyde that has been formed. Thus the oxygen liberated in presence of ethanol under given experimental conditions expressed as percentage of that of the control is a direct measure of the efficiency of peroxidatic oxidation.

By varying the concentrations of catalase (2×10^{-5}–2×10^{-10} M), of ethanol (0·2–8·0 %) and of initial H_2O_2 in the lower chamber ($0{\cdot}25$–$2{\cdot}0 \times 10^{-3}$ M) the following results have been obtained: The efficiency of peroxidatic oxidation *increases* (1) with rising catalase concentration at constant ethanol concentration, reaching 70–80 % with a 2×10^{-5} M catalase; (2) with rising ethanol concentration at constant catalase concentration. It *decreases* with rising H_2O_2 concentration at constant catalase and ethanol concentrations. It is concluded, in agreement with the view of Chance (1949, 1950), that both H_2O_2 and ethanol compete for the H_2O_2-catalase complex I. Peroxidatic as opposed to catalatic activity of catalase is favoured by a low concentration of free H_2O_2 relative to that of ethanol.

The formation of aldehyde, besides its characteristic smell, has been confirmed by a brown decoloration of KOH-soaked filter paper (polymerization) and by Schiff's reagent.

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A Histochemical Study of the Specificity of Phosphatases. By G. H. BOURNE (introduced by F. L. WARREN). (*Department of Histology, London Hospital Medical College, London, E.* 1)

If the ability of alcohol or acetone fixed tissues to hydrolyse various phosphate esters is studied histochemically it can be seen that the distribution of dephosphorylating activity differs with the ester used. The distribution of dephosphorylating activity of sugar phosphates such as those of fructose, sorbose and galactose and also of vitamin K phosphate is similar to that obtained with glycerophosphate and this suggests that these substances are hydrolysed by the non-specific phosphatases.

However, the distribution of dephosphorylating activity for other phosphate esters differs considerably from that obtained with those mentioned above. The other esters studied were riboflavin 5-phosphate, pyridoxal phosphate, aneurin pyrophosphate, tetrasodium 3:4′-dihydroxy-4-methoxychalkone diphosphate, tetrasodium 2′:3-dihydroxy-4:4′-dimethoxychalkone phosphate, oestrone 3-phosphate, tetrasodium stilboestrol diphosphate, tetrasodium hexoestrol diphosphate.

With the latter group of esters the dephosphorylating activity is much greater in the nuclei, and particularly in the nucleoli, than with the first group. In the testis the sperm heads were very positive with the chalkone phosphates.

The hydrolysis of all the esters was tested at pH 9, and at this pH the aneurin pyrophosphate molecule does not remain intact. It is of interest, however, that the distribution of the dephosphorylating activity for the phosphate bearing moiety is quite different from that for glycerophosphate.

The distribution of dephosphorylating activity for the different members of the second group of esters varies with each ester with two exceptions—those for the chalkone compounds are similar in some organs and those for stilboestrol and hexoestrol compounds are also similar but different from that for oestrone phosphate.

This work suggests that there are a number of stable specific dephosphorylating enzymes present in tissues which are different from the non-specific enzyme or enzymes which split glycero- and sugar phosphates and certain other phosphates.

The 'Anti-thrombic' Action of Heparin; The Relation of Sulphydryl Groups. By J. GREEN. (*North Western Group Laboratory, London, N.W.* 3)

Further study of the action of reducing agents on the 'anti-thrombic' action of heparin (Green, 1953) indicates that the findings can be grouped under three headings.

(1) Proportionate retardation of normal and heparinized plasma clotting.

Most reducing agents have this effect when incorporated in plasma. The increase in the apparent thrombin 'bound' by heparin is of the same order as the retardation of normal plasma. Exceptions to this rule, e.g. BAL, may be due to spontaneous oxidation of the reagent, but this is difficult to prove.

(2) True selective retardation of heparinized clotting.

All reducing agents tested have produced this effect when added to thrombin solutions, in concentrations below that at which normal plasma clotting is significantly retarded.

(3) Bizarre anti-heparin effects may develop, particularly when plasma containing BAL, or thrombin solutions with thioglycollate, dithionite or thiol-benzoate are left for a period of time; the stoicheiometric relation between heparin and 'thrombin' no longer obtains. Failure to reproduce this unique anti-heparin effect by oxidation of thioglycollate with H_2O_2 or alloxan, and in air or thrombin solutions, led to the view that it was due to reduction of 'thrombin' (Green, 1953). Incubation of the thioglycollate-thrombin under nitrogen, however, prevents the development of the anti-heparin effect, and on this, and other evidence it must be assumed to be associated with an oxidized form of the above SH reagents. There is a heat-stable factor in commercial thrombin which accelerates the aerobic oxidation of BAL.

The above findings are compatible with the hypothesis that the 'anti-thrombic' action of heparin is due to an effect on a preceding reaction in plasma clotting involving the formation of —S—S bonds, and not on the thrombin-fibrinogen reaction. This is in accord with recent views on plasma clotting (Lorand, 1950; Laki, 1953). Some results with *p*-chloro-mercuribenzoate lend support to this view.

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Taurine in Fresh and Iced Skeletal Muscle of Codling (*Gadus callarias*). By N. R. JONES. (*Torry Research Station, Department of Scientific and Industrial Research, Aberdeen*)

Ethanol (75% v/v) extracts of codling muscle have been chloroform-separated (Awapara, 1948) and subjected to two-dimensional chromatography in phenol-ammonia and collidine-lutidine (Dent, 1948). Chromatograms showed a large ninhydrin-reducing spot, R_F values: phenol, 0·41; collidine, 0·40. This was not attributable to any basic extractive found in codling or the related gadoid haddock (*Gadus aeglefinus*) by separation on sulphonated polystyrene (SPX) columns (Shewan, 1953; Shewan, Fletcher, Partridge & Brimley, 1952).

Pretreatment of chromatograms with $CuCO_3 . Cu(OH)_2$ (Crumpler & Dent, 1949) did not affect the running of the spot, and its detection in effluents from SPX columns indicated an acidic nature. R_F values in different solvents, final isolation from effluents by the method of Campbell & Work (1952), and also direct separation on Dowex 2 columns identified the substance as taurine. Estimated by a spot-elution method (Thompson, 1951) at 400–450 mg./100 g. muscle, taurine is quantitatively among the more important of the nitrogenous extractives. It has been reported in the liver and bile of other fish species (Ackermann & Mohr, 1937; Sihn and his collaborators: Asikari, Kim & Sihn, 1938; Sihn & Maeda, 1938; Sihn & Kim, 1938; Minato & Nisaburo, 1949) in herring (*Clupea harengus*) extractives (Mörner, 1937), and in dried Japanese cod (*Gadus Brandtii*) by Yoshimura & Kanai (1913).

Gutted codling, iced to simulate practical fish-trade conditions, became rapidly depleted of taurine. Some 40 mg./100 g. only remained after 21 days, the greatest rate of loss being over the first 4 days. Non-protein N fell from 380 to 260 mg./100 g. over 21 days.

Incubation of muscle minces and aerated mixed muscle and skin homogenates at 0° led to little change in taurine content over 6 days, although a heavy, typical marine spoilage microflora became established. It appears that the initial rapid drop in taurine in iced muscle probably derives from leaching by ice water.

This work was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

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The Phosphorylation of Pantothenic Acid by *Lactobacillus arabinosus* 17–5. By J. BADDILEY, D. E. HUGHES, A. P. MATHIAS and W. S. PIERPOINT. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Sheffield and Lister Institute, London, S.E.* 1)

In previous papers it was shown that washed suspensions of *Lactobacillus arabinosus* 17–5 would synthesize coenzyme A (CoA) from pantothenic acid only if cystine were added; in the absence of cystine the pantothenic acid was taken up and bound by the cells as a phosphorylated derivative. The phosphorylated bound form was not available for the growth of *Lb. arabinosus*, but pantothenic acid was released quantitatively on treatment with alkaline phosphatase. In the present paper some further studies on the phosphorylation of pantothenate are reported.

In experiments with washed suspensions of intact cells (400 mg. dry wt.) the added pantothenic acid (500 μm-mole) was phosphorylated almost completely in 1 hr. The phosphorylated material was extracted from the cells and examined by paper chromatography and paper electrophoresis, and its behaviour compared with synthetic pantothenic acid 4′-phosphate, pantothenic acid 2′-phosphate, pantetheine and pantetheine 4′-phosphate. In three solvents (*iso*butyric acid/ammonium *iso*-butyrate/water; butanol/acetic acid/water; propanol/ammonium sulphate/water) spots were ob-

tained which contained the bulk of the phosphorylated pantothenate added and whose R_F's corresponded closely to that of pantothenic acid 4'-phosphate. The spots contained no detectable SH groups, but did contain material absorbing strongly at 260 mμ. This absorbing material was separated from the pantothenate by paper electrophoresis in sodium acetate or ammonium formate buffers.

Cells dried over P_2O_5, and extracts of cells broken by crushing at $-25°$ in a bacterial press (Hughes, 1951) would phosphorylate pantothenic acid, only if ATP was present. The amount of phosphorylation was increased by the addition of sodium fluoride (5×10^{-2} M). The stability in acid and alkali of the phosphorylated derivative prepared by dried cells corresponds to that of pantothenic acid 4'-phosphate.

These results suggest that pantothenic acid 4'-phosphate may be formed from pantothenic acid by *Lb. arabinosus*, prior to pantetheine formation. They also lend support to the suggestion of Brown & Snell (1953) that the product formed by incubating dried cells of *Proteus morganii* with ATP, pantothenic acid and cystine might be pantothenyl cysteine-4' phosphate.

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Synthesis of Fatty Acids from Acetate by Preparation of the 'Cell-sap' of Rat Mammary Gland.

By G. Popják* and Alisa Tietz.† (*The National Institute for Medical Research, Mill Hill, London*)

The synthesis of short- and long-chain fatty acids from acetate by cell-free homogenates (suspensions of cell fragments) of rat and sheep mammary gland has previously been reported (Popják & Tietz, 1953*a*). High-speed centrifugal fractionation (at 25000 *g* and 104000 *g*) of homogenates of rat mammary gland yielded a soluble enzyme system, which is essentially the 'cell sap' of mammary gland cells diluted with buffer and which, after suitable additions, synthesizes short- and long-chain fatty acids from [^{14}C]acetate more efficiently than the original homogenates (Popják & Tietz, 1953*b*). The soluble preparations retained their activity after being kept frozen in solid CO_2 for several days. Whereas in the homogenates the addition of either pyruvate, oxaloacetate or α-oxoglutarate and of ATP was necessary to activate fatty acid synthesis from acetate, in the soluble enzyme system the addition of ATP (0·01 M) alone was sufficient for activation. Nevertheless, oxaloacetate and α-oxoglutarate caused even in the soluble preparation a very significant stimulation of fatty acid synthesis. Without added ATP only traces of acetate were incorporated into fatty acids; the optimum concentration of both ATP and of acetate was 0·01 M. The maximum synthesis of fatty acids by the soluble preparations was attained in 70 min. and 50% synthesis in 20 min. Incubation under N_2 caused, as with the homogenates, 80–90% inhibition. Among the various substances tested (2:4-dinitrophenol, 2×10^{-4} M; 2:6-dichlorophenolindophenol, 30 μg./ml.; KF, 0·05 M; NaN_3, 0·01 M; KCN, 0·01 M; Na_3AsO_4, 0·05 M; $HgCl_2$, 1×10^{-4} M; malonate, 0·05 M) arsenate and Hg^{2+} caused 95%, and CN 70% inhibition of synthesis; 2:4-dinitrophenol was also slightly inhibitory. By chromatographic separation of the short- and long-chain fatty acids it was found that after 1 hr. incubation, three times as much ^{14}C was incorporated into the short-chain fatty acids (C_4–C_{10}) as into the long-chain acids (C_{12}–C_{18}).

The preparations of 'cell-sap' took up O_2, and it was found that the oxygen uptake was dependent on the presence of ATP, the highest $-Q_{O_2}$ being observed in the presence of 0·005 M-ATP. Although the addition of substrates, especially of oxaloacetate or of α-oxoglutarate, stimulated O_2 uptake, nevertheless it appeared from experiments in which 2:6-dichlorophenol indophenol or NaN_3 was added to the preparations, that the cytochrome system is not involved in this oxygen uptake although cytochrome *c* is present in the enzyme preparations.

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The Biosynthesis of Riboflavin in *Eremothecium ashbyii*. By T. W. GOODWIN and S. PENDLINGTON. (*Department of Biochemistry, University of Liverpool*)

It is well known that *Eremothecium ashbyii* synthesizes large amounts of riboflavin, most of which is excreted into the culture medium; little is known, however, of the mechanism of synthesis. Using as basal medium containing (% w/v) glucose 1·0; $CaCl_2$, 0·02; $MgSO_4$, $7H_2O$, 0·1; NaCl, 0·02 and KH_2PO_4, 0·2, good growth and riboflavin production can be obtained by the addition of peptone, maximal concentration of riboflavin being achieved with 0·02 % (w/v) peptone N (compare Schopfer, 1944) and maximal yield with 0·04 %. Under these conditions growth is complete within 5 days, but riboflavin continues to be produced up to 8 days after inoculation.

The effect of amino acids on flavogenesis was examined by adding the amino acid under test at a level of 0·020 % N to the basal medium containing 0·005 % peptone N and comparing the results with those obtained using the basal medium containing 0·025 % peptone N. The amino acids fell into three groups: (*a*) those that had no effect on either growth or riboflavin synthesis—most of those tested; (*b*) those that stimulated both growth and flavogenesis equally—asparagine, aspartic acid, glutamic acid; and (*c*) those which stimulated riboflavin synthesis considerably without having any effect on growth—serine, threonine and (to a lesser extent) tyrosine. The optimum concentration of threonine N for riboflavin stimulation was eventually found to be 0·001 %; the D-isomer was inactive.

Various purines and pyrimidines were tested under the same conditions, and it was found that whilst adenine and xanthine (and adenosine) strongly stimulated riboflavin synthesis without affecting growth, the pyrimidines were in general without effect on either growth or flavogenesis. High levels of uracil slightly inhibited riboflavin synthesis. These observations confirm those of Maclaren (1952) made under different conditions.

The stimulatory effects of threonine, serine and tyrosine are not cumulative, whereas those of a purine plus one of these amino acids are cumulative.

A tentative conclusion from these results is that the amino acids provide no N for the biosynthesis of riboflavin but do provide C units for the synthesis of its aromatic ring, whilst the purines provide the remainder of the *iso*alloxazine structure. These ideas are being investigated using isotopes.

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The Arylsulphatase of an *Alcaligenes* Isolated from a Marine Mud. By K. S. DODGSON, T. H. MELVILLE, B. SPENCER and K. WILLIAMS. (*Physiology Institute and Department of Botany, University of Wales, Cardiff*)

Karunairatnam & Levvy (1951) showed the β-glucuronidase activity of sheep rumen to be due to bacteria. It was possible that the high arylsulphatase activity of marine molluscs (Dodgson, Lewis & Spencer, 1953) was of similar origin. Bacteria isolated from homogenates of the visceral humps of a number of molluscs showed no arylsulphatase activity when grown under varying conditions on plates containing 0·001 M tri-potassium phenolphthalein disulphate (cf. Barber, Brooksbank & Kuper, 1951). Certain agar-digesting bacteria isolated from the red seaweed *Corallina* and other organisms isolated from several intertidal muds had no arylsulphatase. However, two micro-organisms present in intertidal mud collected at Sully, Glamorgan, showed considerable enzyme activity. One of these organisms was identified as a yeast, *Trichosporon cutaneum* and the other as a bacterium of the genus *Alcaligenes*. These arylsulphatase producers appeared to be localized in this particular area, since they were not found in mud samples taken from other parts of the Bristol Channel. Greatest activity was found in *Alcaligenes*, two-thirds of the total activity of the culture being associated with the bacterial mass. No increase in enzyme activity occurred when the organism was grown in the presence of potassium *p*-acetylphenyl sulphate.

Acetone-dried concentrates of the bacterial mass were maximally active at pH 8·75 in 0·1 M phosphate when the concentration of potassium *p*-acetylphenyl sulphate was 0·003 M, the enzyme being inhibited by excess of substrate (contrast the findings for the arylsulphatase of *Myco. piscium*; Whitehead, Morrison & Young, 1952). The high optimum pH was not an artifact arising from the acetone

treatment since an aqueous extract of the crushed bacteria behaved similarly. When potassium *p*-nitrophenyl sulphate (0·006M in 0·1M phosphate) was the substrate the optimum pH was also 8·75. The high optimum pH of this enzyme is interesting in view of the ability of *Alcaligenes* to produce alkali. During cultivation the pH of the medium increased from 7·5 to 8·7.

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A Method for the Isolation of Urinary Mucoproteins. By A. J. ANDERSON. (*Department of Chemical Pathology, Westminster Medical School, London, S.W.* 1)

At the moment two methods are available for the isolation of urinary mucoproteins. Both are precipitation reactions, one with 0·58M sodium chloride (Tamm & Horsfall, 1952) and the other with ethanol (Tamm & Horsfall, 1950). Both methods, however, suffer from certain disadvantages. The yield is low with the first method while with the second method the large amount of ethanol needed is a drawback. The following method relies on the adsorption of the mucoproteins on benzoic acid.

A 20% (w/v) acetone solution was added to normal male urine and the precipitate obtained treated with acetone, the residue remaining being treated with water at pH 9–9·5. After exhaustive dialysis the mucoprotein was precipitated by the addition of sodium chloride to 0·2M, the solution then being poured into five volumes of chilled acetone. A white powder (mucoprotein A) was obtained: yield 2·17 g. from 56 l. of urine (3·87 mg./100 ml. urine). Ethanol precipitation of the supernatant yielded a brown powder (mucoprotein B). Yield 256 mg. from 8·25 l. of urine (3·11 mg./100 ml. urine).

Both mucoproteins were readily water soluble and neither could be precipitated by the usual protein precipitants. Both gave a positive Molisch reaction. Mucoprotein A gave a positive biuret, ninhydrin, xanthoproteic, Millon and Sakaguchi reaction. Mucoprotein B gave a negative biuret and ninhydrin, the other reactions being either negative or very weakly positive. The Selivanoff and Bial tests for ketosugars and pentoses, respectively, were negative, as was the aldehyde test for tryptophan. Analysis (mucoprotein A and B, respectively): N, 10·54, 5·10%; acetyl (Kuhn & Roth, 1933), 3·67, 9·03%; hexosamine (Tracey, 1952), 14·41, 18·29%; reducing power expressed as glucose (before hydrolysis), 7·2, 3·6%; reducing power (the maximum liberated after hydrolysis with 2N-HCl at 100°), 17·5, 30·7%. The Morgan & Elson (1934) test for *N*-acetylhexosamine was positive for both proteins. It was also positive for the diffusate obtained on dialysis of the protein hydrolysates.

By the usual methods of paper chromatography (Consden, Gordon & Martin, 1944) the following amino acids were identified for both mucoproteins: aspartic acid, glutamic acid, phenylalanine, tyrosine, valine, proline, arginine, lysine, alanine, threonine, glycine, serine, leucine (*iso*leucine). Galactose, mannose and fucose were identified (Jermyn & Isherwood, 1949) and also glucosamine (Partridge, 1948). A second hexosamine, probably galactosamine, was present.

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Enzymic 11-β-Hydroxylation of Progesterone by Ox Adrenocortical Mitochondria. By A. C. BROWNIE and J. K. GRANT. (*Biochemistry Department, University of Edinburgh*)

It has been suggested (Hechter, Zaffaroni, Jacobsen, Levy, Jeanloz, Schenker & Pincus, 1951) that progesterone is a key intermediate in the biosynthesis of the adrenocortical hormones. The determination of the course of metabolism of this steroid in the adrenal gland would thus appear to be of importance. Perfusion of progesterone through ox adrenals gave a variety of hydroxylated derivatives, of which 11-β-hydroxyprogesterone was obtained in less than 1% yield (Hechter *et al.* 1951). Incubation

of progesterone and of 17-α-hydroxyprogesterone with whole adrenal homogenates gave corticosterone and 17-α-hydroxycorticosterone respectively, but no 11-β-hydroxyprogesterone (Hayano & Dorfman, 1952). With washed adrenal homogenate residues, progesterone and 17-α-hydroxyprogesterone were inactive as substrates (Hayano & Dorfman, 1953). This observation led to the suggestion by these authors that 11-β-hydroxylation does not occur in the absence of a ^{21}C hydroxyl.

In the present work progesterone (45 mg.) was incubated aerobically for 1 hr. with ox adrenocortical mitochondria (400 mg. wet wt. mitochondria per 0·5 mg. progesterone) in a medium containing phosphate buffer (pH 7·4, 0·04 M), Mg^{2+} (0·005 M), KATP (0·0005 M) and K succinate (0·01 M). Progesterone was determined by absorption at 240 mμ., following recovery from incubation mixtures in 95 ± 3% yield, by solvent partition procedures. About half of the progesterone incubated was metabolized. From the products 8 mg. crystalline 11-β-hydroxyprogesterone was isolated by procedures involving seven-stage solvent distribution with double withdrawal (Craig & Craig, 1950) followed by chromatography on alumina and recrystallization. The product was identified by m.p. and mixed m.p. with authentic 11-β-hydroxyprogesterone, absorption spectra of a solution in sulphuric acid and by i.r. spectra.

As in the case of DOC (Brownie, Grant & Taylor, 1953) 11-β-hydroxylation of progesterone requires the concurrent oxidation of Krebs cycle acids. However, on account of the greater inhibition of the operation of the Krebs cycle in adrenocortical mitochondria by progesterone as compared with DOC 11-β-hydroxylation of progesterone could only be achieved with a suitably increased enzyme/steroid ratio.

These results show that preliminary 21-hydroxylation of progesterone is not necessary for 11-β-hydroxylation.

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Liver Catalase Activity in the Riboflavin Deficient Mouse. By D. H. Adams. (*Cancer Research Department, London Hospital Medical College, London, E.* 1)

There is a sex difference in mouse-liver catalase activity, males having the higher level. Castration of males results in a fall in enzyme activity, and the injection of testosterone into castrated males or normal females elevates their catalase activity to the normal male level. Adrenalectomy of normal females or castrated males produces a fall in catalase activity which is reversed by the injection of cortisone. Testosterone and cortisone appear to act independently on catalase (Adams, 1952).

The effect of riboflavin deficiency is now being studied in an attempt to gain more information about the normal mechanisms controlling liver catalase activity. Mice have been kept on a riboflavin-free diet, and catalase activity determined at intervals. As always in experiments of this nature two separate effects are involved: first, riboflavin deficiency, and secondly, the associated fall in total food intake. When weanling male mice are placed on a riboflavin-free diet, catalase levels tend to fall in both the deficient group and the pair-fed group receiving riboflavin, instead of rising to the higher adult level. This may be due to an interference with testicular development due to the lowering of food intake. If, however, the mice are injected with testosterone after 4–5 weeks on the diets, a rise in liver catalase activity to the normal adult level is obtained in the pair-fed controls, but not in the riboflavin deficient group. The dietary intake of weanling female mice kept under the same conditions is somewhat higher than in the males, and female catalase level remains normal in riboflavin-deficient, pair-fed, and *ad lib.* normal diet groups. Again, however, the catalase activity of the riboflavin deficient animals is not raised by testosterone, while the other groups react normally. In males continuously injected with testosterone after being kept for 3 weeks on the diets, catalase level rises in pair-fed groups and continues to fall in riboflavin deficient groups. If riboflavin is then given to the deficient animals (testosterone still being injected, and total dietary intake being held at the same level), their catalase activity rises to normal.

It appears therefore that testosterone will not affect catalase level in the riboflavin deficient mouse. The normal catalase level in riboflavin deficient females suggests that cortisone does not require the presence of riboflavin for its action on catalase.

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DEMONSTRATIONS

Porphyrins in the Urine of Normal Men. By A. COMFORT and M. WEATHERALL. (*Department of Pharmacology, London Hospital Medical College, London, E.* 1)

A Histochemical Study of the Specificity of Phosphatases. By G. H. BOURNE (introduced by F. L. WARREN). (*Department of Histology, London Hospital Medical College, London, E.* 1)

Light Scattering Apparatus By R. A. GRANT. (*Department of Biochemistry, London Hospital Medical College, London, E.* 1)

A relatively simple apparatus has been developed for the determination of weight-average molecular weights of proteins and for use in kinetic studies of macromolecular systems.

Paper Ionophoresis of Phosphoric Esters. By M. W. NEIL and D. G. WALKER. (*Department of Biochemistry, London Hospital Medical College, London, E.* 1)

Turba & Enenkel (1951) separated adenosine and its phosphates by paper electrophoresis in acetate buffer at pH 4. Schild & Maurer (1951) prepared a haemolysate of human red blood cells, which had been incubated with a saline solution containing radioactive phosphate ions. Small amounts of the preparation were placed on filter paper strips which were then wetted with veronal buffer, pH 8·6. After applying a p.d. across the ends of the strips for a period of time, they found that the distribution of radioactivity along the paper was concentrated in certain regions.

The technique of paper ionophoresis has now been applied in particular to the problem of separating mixtures of inorganic phosphates and triose and hexose phosphates. Phosphoric acid esters exist as anions in solution and move towards the anode in a variety of buffer solutions and over a wide range of pH values when a p.d. is applied across the ends of a strip of filter paper, wetted with buffer solution, on which the esters have been placed. Differences of pH with one particular buffer, and different buffers at the same pH, affect the rate of flow of the esters to a marked extent. Complete separations of all the esters have not been achieved, e.g. the hexose monophosphates run at almost identical rates under all the conditions tested. However, the ionophoresis technique has an advantage over paper chromatography (Walker & Warren, 1951) in that useful fractionations of tissue extracts may be achieved directly without preliminary treatment. The more classical methods and enzymic methods may then be applied to provide further more detailed analytical data. A scheme is proposed whereby such analyses may be undertaken.

Various methods for paper ionophoresis are demonstrated, together with some results obtained by such means.

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The Separation of Sugars and of Sugar Phosphates by Gradient Elution from Ion Exchange Columns. By C. W. PARR. (*Department of Biochemistry, London Hospital Medical College, London, E.* 1)

Mixtures of monosaccharides, after adsorption as borate complexes on strong-base anion-exchange resins, have been separated and analysed by elution with weak borate solutions (Khym & Zill, 1951, 1952; Noggle & Zill, 1952). By eluting with mixtures of chloride and borate, hexose phosphates have more recently been resolved (Khym & Cohn, 1953).

The method to be demonstrated, while based on similar principles, was evolved independently of the work quoted above, and differs in many respects.

A fine grade of the strongly basic anion-exchange resin De-Acidite FF (Permutit Co. Ltd., London) is used and this is packed, resting on a sintered glass disk, in a glass column of internal cross-sectional area 1 cm.^{2}, so that the length of the resin bed is 10 cm. The resin is prepared in the borate form and mixtures of free sugars and hexose phosphates (up to a total of about 50 mg.) in aqueous solution are passed through the column and adsorbed.

Sucrose, fructose, glucose, glucose 1-phosphate, glucose 6-phosphate, fructose 6-phosphate and fructose 1:6-diphosphate in the same mixture may be resolved by the following process of gradient elution: two identical reservoirs *A* and *B* (1 l. aspirator bottles) are kept at identical horizontal levels and are filled, *A* with 1 l. of 0·1 M boric acid adjusted to pH 7·0 with sodium hydroxide, and *B* with 1 l. of 0·1 M boric acid at pH 7·0 but containing also 0·25 M sodium chloride. The reservoirs are connected by a narrow tube so that, as boric acid flows from reservoir *A* (which is stirred electrically) into the column, so boric acid containing chloride flows from reservoir *B* to reservoir *A*, and the levels of liquid in *A* and *B* remain the same. With this apparatus the chloride concentration of the eluting solution, which is initially zero, increases linearly with the volume eluted, and becomes 0·25 M for a volume of 2 l. Portions (10 ml.) are collected, using an automatic fraction collector, and the various fractions are located using, in particular, the quantitative anthrone method described by Trevelyan & Harrison (1952).

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Photometry of Dyed Material in Filter Paper. By E. M. Crook, H. Harris, F. Hassan and F. L. Warren. (*Departments of Biochemistry, University College London and London Hospital Medical College*)

Genetics of Cystinuria. By H. Harris, Ursula Mittwoch, Elizabeth B. Robson and F. L. Warren. (*Galton Laboratory, University College London and Department of Biochemistry, London Hospital Medical College*)