PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **299th Meeting** *of the Biochemical Society was held in the Department of Biochemistry, University of Sheffield, Sheffield* 10, *on Saturday,* 21 *July* 1951, *when the following papers were read:*

COMMUNICATIONS

The Metabolism of Azobenzene and *p*-Hydroxyazobenzene in the Rabbit. By H. G. Bray, R. C. Clowes and W. V. Thorpe. (*Department of Physiology, Medical School, University of Birmingham*)

The fate of the azo (—N=N—) group in the animal body is of interest because rupture of this linkage may give rise to compounds with conjugable centres so that the azo group could act as a 'potential centre for conjugation' (Bray, Ryman & Thorpe, 1948). Failing rupture of the azo group, conjugation with glucuronic acid or sulphuric acid could be achieved by introduction of a new hydroxyl group by hydroxylation. Previous workers have shown that the linkage can be broken, e.g. in azobenzene by the rat (Elson & Warren, 1944), and in *p*-dimethylaminoazobenzene by the rat (Stevenson, Dobriner & Rhoads, 1942; Miller, Miller & Baumann, 1945). Hydroxylation of the intact azobenzene molecule has not been observed.

In the present investigation an attempt has been made to assess the extent of the cleavage of the —N=N— linkage and degree of hydroxylation of azobenzene and some of its derivatives in the rabbit. Azobenzene is not completely absorbed, about 30 % of a dose of 0·5 g./kg. being found in the faeces. Approximately 60 % of the absorbed azobenzene was excreted as glucuronide and 20 % as sulphate (these values calculated on the basis of 1 mol. conjugating acid/mol. azobenzene) and 23 % unchanged. Corresponding values for *p*-hydroxyazobenzene, which was completely absorbed, were: unchanged, 3 %; glucuronide, 95 %; and sulphate, 27 %. The following metabolites have been identified in the urine after administration of azobenzene (figures in parentheses indicate percentage of dose isolated, other compounds were detected by paper chromatography): excreted free, *p*-hydroxyazobenzene (1·5), *o*-hydroxyazobenzene, benzidine (2), *p*-acetamidophenol, *o*-acetamidophenol, hydrazobenzene; excreted conjugated with glucuronic or sulphuric acids, *p*-hydroxyazobenzene (3), benzidine, *p*-aminophenol (5), *o*-aminophenol. The following were found after administration of *p*-hydroxyazobenzene: excreted free, *p*-acetamidophenol, *o*-acetamidophenol; and conjugated, *p*-aminophenol (57), *o*-aminophenol. Other derivatives of azobenzene are being examined.

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The Metabolism of 2:3:5:6-Tetrachloronitrobenzene and 2:3:4:5-Tetrachloronitrobenzene in the Rabbit. By H. G. Bray, Z. Hybs, H. J. Lake and W. V. Thorpe. (*Department of Physiology, Medical School, University of Birmingham*)

2:3:5:6-Tetrachloronitrobenzene (TCNB) is only absorbed to a small extent by the rabbit. With doses from 0·1 to 3·0 g., 60–80 % can be recovered unchanged from faeces; even a dose of 10 mg. is not completely absorbed. The TCNB which is absorbed is mainly excreted as glucuronide and mercapturic acid (12 and 7 % of dose respectively). Tetrachloroaminophenol (m.p. 186°) and the mercapturic acid (m.p. 207°) have been isolated from the urine. No toxic effects were observed.

The asymmetrical isomer of TCNB, 2:3:4:5-tetrachloronitrobenzene, caused anorexia in doses of 0·7 g. Absorption is greater than with the symmetrical isomer, only one-third of a dose being found in the faeces. A considerable part of the unabsorbed material is reduced in the gut to the amine which, unlike that of TCNB, readily diazotizes and couples. The absorbed compound is partly converted into glucuronide and ethereal sulphate (27 and 7 % of dose respectively). 2:3:4:5-Tetrachloroaniline was isolated from the urine (m.p. 118–120°). No mercapturic acid formation was detected.

***Leuconostoc citrovorum* Factor and the Synthesis of Serine by Micro-organisms.** By JUNE LASCELLES, M. J. CROSS and D. D. WOODS. (*Department of Biochemistry, University of Oxford*)

It has been shown (Lascelles & Woods, 1950) that pteroylglutamate and pyridoxal are essential for the synthesis of serine from glycine and formate (in the presence of glucose) by washed cells of *Streptococcus faecalis* R deficient in these factors. Formylpteroylglutamate was 30% more active than pteroylglutamate. *Leuconostoc citrovorum* factor is replaced for growth of that organism by a synthetic tetrahydro derivative of formylpteroylglutamate ('leucovorin') (Brockmann, Roth, Broquist, Hultquist, Smith, Fahrenbach, Cosulich, Parker, Stokstad & Jukes, 1950), which was therefore tested in the above system. A sample of this material was kindly provided by Dr T. H. Jukes. It replaced pteroylglutamate, but was not more active when added simultaneously with the other components. However, cells first incubated in glucose-phosphate with leucovorin synthesize serine more actively than cells similarly treated with pteroylglutamate when tested in the serine synthesis system with or without pteroylglutamate.

When serine synthesis in the above systems is promoted by pteroylglutamate it is accompanied by formation of *L. citrovorum* factor. Such synthesis requires only formate, glucose and pteroylglutamate; the concentration of formate necessary is less than that required for serine synthesis.

It was also found (Lascelles & Woods, 1950) that the serine requirement for growth of *Leuconostoc mesenteroides* could be replaced by high concentrations of glycine provided that pyridoxal was present and the atmosphere was enriched with CO_2. It is now found that there is a quantitative relationship between concentration of glycine and partial pressure of CO_2, which suggests that, with growing cultures, CO_2 may act as precursor of the one-carbon unit condensed with glycine or a derivative. Leucovorin replaces the requirement for added CO_2; the low concentration necessary makes it impossible that the formyl group of this compound serves directly as source of the one-carbon unit.

Leucovorin also replaces *p*-aminobenzoic acid for growth of *L. mesenteroides* in either the presence or absence of serine. It overcomes sulphonamide inhibition of growth in a non-competititive manner. Neither pteroylglutamate nor its formyl derivative is active in these respects.

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Adaptation of *Bacillus subtilis* to Fatty Acids. By H. LASER. (*Molteno Institute, University of Cambridge*)

Vaccenic acid

$$[CH_3.(CH_2)_5.CH{=}CH.(CH_2)_9.COOH],$$

which occurs in, and has been isolated from, a number of animal organs (Laser, 1949; Morton & Todd, 1950), has previously been shown to inhibit growth and respiration of *Bacillus subtilis* (Laser, 1950, 1951). The inhibition is transient at suitable concentrations of acid, respiration and growth ultimately attaining the same, or slightly higher, rates than those of untreated controls. Organisms which have recovered from the inhibition are resistant to further addition of acid. This phenomenon, which resembles acquired drug resistance by bacteria, has been further studied on cells treated with stearic, oleic, vaccenic and linoleic acid. The main results are as follows: (1) The acids tested inhibit growth and respiration qualitatively in a similar manner, but quantitatively in different degrees (stearic acid < oleic acid < vaccenic acid < linoleic acid). (2) At suitable concentrations recovery from the inhibition occurs with time, both in growing cells and in washed suspensions. (3) Recovered cells are resistant to considerably higher concentrations of acid than the initially inhibiting dose. (4) Treatment with any one acid produces resistance against any of the other acids. (5) A time lag ensues on treatment with a fatty acid before resistance occurs. (6) Low concentrations of acid, which do not inhibit growth or O_2 uptake, nevertheless produce resistance. (7) The tolerance of growing cells with subsequent development of resistance is slightly greater than that of washed suspensions. (8) The resistance is lost on subculturing the micro-organisms in absence of added fatty acid.

The evidence suggests that the resistance of treated cells is due to the development of an adaptive enzyme, which is formed subsequently to the

incorporation into the cell and metabolic degradation of the fatty acid. It therefore appears possible that the adaptive enzyme is produced by a mechanism resembling an immunological reaction, i.e. in response to the formation of a conjugated antigen, which arises from the intracellular combination of a preformed protein with a partial degradation product of the substrate.

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Effect of Carbon Monoxide on the Level of Energy-rich Phosphate in Surviving Muscle.
By R. A. Lawrie. (*Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge*)

The effect of carbon monoxide (CO) on the energy-rich phosphate ($\sim$P) levels of pre-rigor horse psoas muscle has been studied. For this purpose $\sim$P was taken to be the sum of the labile phosphorus of creatine phosphate and adenosinetriphosphate. The latter value was obtained by correcting '$P_{7\ min.}$' for triose- and hexose-diphosphates. Inorganic phosphate was estimated by the method of Allen (1940), after controlled hydrolysis of the various fractions. A series of gas mixtures, representing selected $CO:O_2$ ratios, was prepared, and to these were exposed, in sealed vessels at room temperature, thin layers (2–3 mm.) of minced horse psoas muscle from the first to the seventh hour post-mortem. The minces were then extracted by ice-cold trichloroacetic acid (TCA), the extracts being immediately neutralized. Control TCA extracts were made simultaneously from samples exposed to corresponding mixtures containing $N_2:O_2$. The $\sim$P content of these extracts was expressed as percentage total soluble phosphorus and the effect of CO represented as the difference between test and control values. In the dark, test samples at $CO:O_2$ ratios between 10:90 and 80:20 were found to have higher $\sim$P levels than those in corresponding $N_2:O_2$ controls, the difference being most marked when $CO:O_2$ was about 20:80. When the concentration of CO was greater than 0·8 atm. the $\sim$P level of the test extract dropped considerably below that of the corresponding control. (In 100% CO, however, the $\sim$P value was virtually the same as that in 100% N_2.) A similar but less marked drop occurred when the CO concentration was less than 0·1 atm. The stimulatory effect is no longer evident on illumination.

In the hope of accentuating this phenomenon during conditions when post-mortem glycolysis is greatly accelerated, an analogous series of analyses was performed on pairs of TCA extracts prepared from psoas muscle homogenates, 1 hr. post-mortem, through which had been passed standardized quantities of each $CO:O_2$ or $N_2:O_2$ mixture, for 2 min. before TCA addition. Once again the above effects of CO were apparent, being rather more marked than in the minces.

Glycogen and pH determinations suggest that the rise in $\sim$P is related to increased respiration. Partial inactivation of cytochrome oxidase is presumably responsible for the depression of $\sim$P levels at high CO tensions. The relation of these findings to the Pasteur effect, and to the hypothetical Pasteur enzyme, was briefly discussed.

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Vitamin B_{12} Activity for Chicks and different Micro-organisms of Gut Contents and Faeces.
By M. E. Coates, J. E. Ford, G. F. Harrison, S. K. Kon, J. W. G. Porter (*National Institute for Research in Dairying, University of Reading*), W. F. J. Cuthbertson and H. F. Pegler (*Glaxo Laboratories Ltd., Greenford, Middlesex*)

In our laboratories tests for vitamin B_{12} with *Escherichia coli* (*Bacterium coli*) (Bessell, Harrison & Lees, 1950) and *Lactobacillus leichmannii* ATCC, 4797 (Skeggs, Nepple, Valentik, Huff & Wright, 1950) agree between themselves for a variety of natural materials. Assays of aqueous extracts (prepared by steaming for 30 min. at pH 5 and filtration) of gut contents and faeces of calves and chicks receiving diets containing little or no vitamin B_{12} form a notable exception. Thus tests with fresh calf

faeces gave results of 0·36 μg. vitamin B_{12}/g. with *Esch. coli* and 0·07 μg. with *Lb. leichmannii*. The addition of cyanide (cf. Wijmenga, Veer & Lens, 1950) during extraction increased the results to 1·9 and 0·19, probably by facilitating extraction, since the potency of the aqueous extracts themselves so treated remains unchanged. Accordingly, cyanide treatment was used for all the extracts tested microbiologically. Tests with fresh chick droppings and chick caecal contents gave results with *Esch. coli* of 1·0 and 7·0, and with *Lb. leichmannii* of 0·27 and 1·65 μg. vitamin B_{12}/g., respectively.

A test with *Euglena gracilis* gave a value for dried rumen contents of 3·6, which agreed with the result of 3·8 with *Esch. coli*; the result with *Lb. leichmannii* was 1·1 μg. vitamin B_{12}/g.

Chick tests, which normally give in our experience values higher than the microbiological (Coates, Harrison & Kon, 1951), yielded a result of 0·2 for the dried rumen contents. A sample of dried calf faeces gave results of 10·6 and 1·9 with *Esch. coli* and *Lb. leichmannii*, and 0·4 μg. vitamin B_{12}/g. with chicks. These low values are not due to any depression of the growth of the chicks by the materials tested.

The activity measured by both *Esch. coli* and *Lb. leichmannii* is largely cell-bound in the original material but completely ultrafiltrable in the extracts, where it is destroyed by autoclaving at 15 lb. for 15 min. at pH 11·6. Satisfactory recoveries of added vitamin B_{12} were obtained from extracts before and after alkali treatment.

E. coli was grown in an extract of calf faeces. The harvested cells contained approximately the original ratio of activities for *Esch. coli* and *Lb. leichmannii*.

A method based on the *Esch. coli* cup-plate assay showed that the diffusion coefficients of vitamin B_{12} and the factor in faeces active for *Esch. coli* were approximately the same.

Work on the microbiological, biological and chemical aspects of the problem is continuing.

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The Effect of Antibiotics on the Growth of Chicks deprived of Vitamins of the B Complex.

By M. E. Coates, C. D. Dickinson, G. F. Harrison and S. K. Kon. (*National Institute for Research in Dairying, University of Reading*)

It was shown by Stokstad & Jukes (1950) and confirmed by us (Coates, Harrison, Kon, Mann & Rose, 1951) that antibiotics stimulate the growth of chicks on diets adequate in known vitamins.

To study the effect of the drugs in deficiencies of the B vitamins, groups of 12–16 day old Rhode Island Red × Light Sussex cockerel chicks received the deficient diets of Coates, Kon & Shepheard (1950) either alone or supplemented with penicillin (2·5 mg./100 g.) as the procain derivative. The experiments lasted 4 weeks, or less if high mortality intervened.

The mean final body weights (g.), with the numbers of surviving birds in parentheses, were at 4 weeks: complete diet 340 (15), with penicillin 370 (15); without biotin 311 (15), without biotin, with penicillin 348 (15); without nicotinic acid 225 (15), without nicotinic acid, with penicillin 200 (15); at 3 weeks: without folic acid 104 (13), without folic acid, with penicillin 164 (13); without pantothenic acid 76 (8), without pantothenic acid, with penicillin 84 (9); without riboflavin 70 (13), without riboflavin, with penicillin 73 (15); at 2 weeks: without pyridoxin 56 (8), without pyridoxin, with penicillin 67 (13). Birds receiving no aneurin, with and without penicillin, were all dead by the 12th day.

No vitamin B_{12} was added, since our normal birds usually carry reserves of this factor (Coates, Harrison & Kon, 1949), but further tests were done, in two stages, with its addition at the rate of 2 μg./100 g. diet. The results were, in the first stage, at 4 weeks: complete diet 328 (12), with penicillin 404 (11), with vitamin B_{12} 357 (12), with penicillin and vitamin B_{12} 381 (12); without biotin 254 (9), without biotin, with penicillin 286 (10), without biotin, with vitamin B_{12} 250 (11), without biotin, with penicillin and vitamin B_{12} 272 (11); without nicotinic acid 268 (11), without nicotinic acid, with penicillin 205 (11), without nicotinic acid, with vitamin B_{12} 199 (12), without nicotinic acid, with penicillin and vitamin B_{12} 216 (12); at 3 weeks: without folic acid 108 (3), without folic acid, with penicillin 143 (11), without folic acid, with vitamin B_{12} 102 (4), without folic acid, with penicillin and vitamin B_{12} 189 (11). In the second stage, at 3 weeks: complete diet 236 (16), with

penicillin 228 (16), with vitamin B_{12} 240 (15), with penicillin and vitamin B_{12} 243 (16); without pantothenic acid 70 (7), without pantothenic acid, with penicillin 68 (10), without pantothenic acid, with vitamin B_{12} 79 (8), without pantothenic acid with penicillin and vitamin B_{12} 82 (5); without riboflavin 59 (10), without riboflavin, with penicillin 71 (15), without riboflavin, with vitamin B_{12}, 68 (12), without riboflavin, with penicillin and vitamin B_{12} 71 (15); at 2 weeks: without pyridoxin 52 (3), without pyridoxin, with penicillin 64 (5), without pyridoxin, with vitamin B_{12} 53 (2), without pyridoxin, with penicillin and vitamin B_{12} 61 (4). Birds receiving no aneurin, with and without penicillin and vitamin B_{12}, were all dead by the 11th day.

The vitamins studied fell into three classes. The presence of the antibiotic had no effect on the degree of deficiency of aneurin, riboflavin, pyridoxin or pantothenic acid, lessened that of biotin or folic acid and increased that of nicotinic acid. Although there were indications that the diets were slightly deficient in vitamin B_{12}, the effects of penicillin were essentially the same when vitamin B_{12} was added. On two occasions growth on the supposedly complete diet was improved by addition of the antibiotic, but on the third occasion this improvement was not noted in a 3 week test.

The tendency to lesser mortality among the birds on the diets containing the antibiotic supports the theory that the drug acts by combating a minor infection, but the results with biotin, folic acid and nicotinic acid give strong evidence in favour of its altering the microbial population of the gut. That vitamin B_{12} also increases the severity of nicotinic acid deficiency suggests that it also, in this instance, may encourage the growth of organisms that use nicotinic acid at the expense of the host.

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The Formation of Fatty Acids by a Gram-negative Coccus. By S. R. Elsden, F. M. C. Gilchrist, D. Lewis and B. E. Volcani.* (*Department of Bacteriology, University of Sheffield, and the Division of Plant Nutrition, University of California, Berkeley*)

A strictly anaerobic Gram-negative coccus, isolated from the rumen of sheep, ferments lactate, glucose, fructose, sorbitol and mannitol with the production of volatile fatty acids and gas. The fermentation products from lactate were H_2 and CO_2, acetate, propionate, *n*-butyrate and *n*-valerate with traces of *n*-caproate (hexanoate). Glucose yielded H_2 and CO_2, acetate, *n*-butyrate and *n*-caproate with small amounts of propionate and *n*-valerate.

Washed suspensions of the organism suspended in phosphate buffer (pH 6·5) and under a gas phase of N_2 produced a gas other than CO_2, presumably H_2, from glucose, fructose, sorbitol, mannitol, lactate, pyruvate, formate, DL-alanine and DL-serine. Towards the end of the fermentation of pyruvate there were signs of a gas uptake; and when pyruvate was fermented under an atmosphere of H_2 this secondary uptake of gas was much increased, the pressure returning almost to its initial value by the end of the experiment. Addition of either acetate, propionate, or *n*-butyrate to the fermentation of pyruvate under H_2 increased the utilization of this gas. Analysis of the fermentation fluids for fatty acids by both paper chromatography (Brown, 1950; Hiscox & Berridge, 1950) and the quantitative liquid-gas partition chromatogram of James & Martin (1951) showed that the fermentation of pyruvate yielded acetate, *n*-butyrate and *n*-caproate with small amounts of propionate and *n*-valerate. Addition of acetate about doubled the yields of *n*-butyrate, *n*-valerate and *n*-caproate; addition of propionate gave a tenfold increase in the amount of *n*-valerate; and addition of *n*-butyrate increased the yield of caproate sixfold. There was at the same time a disappearance of the added fatty acid. These results suggest that a C_2 fragment from pyruvate combines with the added fatty acid and the resulting compound is then reduced by the hydrogenase system. These observations are in agreement with the work of Stadtman & Barker (1949) in which it was shown that extracts of a different organism, *Clostridium kluyveri*, converted a mixture of acetyl phosphate and acetate to butyrate and caproate in the presence of H_2.

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The Metabolism of Creatine by a Pseudomonad. By G. Appleyard (introduced by D. D. Woods).
Department of Biochemistry, University of Oxford)

Nimmo-Smith (1949) isolated an organism, provisionally identified as *Pseudomonas eisenbergii*, which can produce an adaptive system of enzymes enabling it to grow on creatine as sole source of carbon and nitrogen. Washed suspensions of organisms grown on a creatine medium were able to remove creatine; but they could not degrade any other tested guanidine derivative, except agmatine and arginine, which were attacked relatively slowly.

It is now found that washed cell suspensions rapidly oxidize creatine. The creatine removal and oxygen uptake continue at a uniform rate until the creatine has completely disappeared. At this stage the oxygen consumption falls, but never to the value without substrate. One molecule of urea is formed per molecule of creatine removed. The oxygen uptake and ammonia and carbon dioxide production (at the stage when creatine has disappeared) are about 55, 30 and 45 %, respectively, below the theoretical values for complete oxidation of the creatine, but no other product has been found. The occurrence of oxidative assimilation is indicated by the fact that these deficits are reduced about 50 % in the presence of azide.

Sarcosine is also oxidized by cell suspensions. At low concentrations the rate of oxygen uptake is only about 20 % of that with equimolar creatine. Raising the sarcosine concentration increases the rate, until it equals that with optimal concentrations of creatine.

Under anaerobic conditions, cell suspensions remove creatine with the formation of one mole of urea; chromatographic evidence suggests that the other product is sarcosine and estimations with an organism which oxidizes this substance indicate that one mole is formed. At the creatine concentration normally used, creatine is removed anaerobically at only about 20 % of the aerobic rate; by increasing the creatine concentration, the anaerobic rate of removal may be brought up to the optimal aerobic rate.

A soluble cell-free enzyme can be obtained by acetone-drying the cells. This enzyme hydrolyses creatine to one molecule of sarcosine (evidence as above) and one molecule of urea. Evidence has been obtained that the enzyme requires the presence of —SH groups for its activity.

It is suggested that the first stage in the aerobic breakdown of creatine by this organism is a hydrolysis to sarcosine and urea. The fact that, at low substrate concentrations, the rate of aerobic removal is considerably greater than the anaerobic rate suggests the possibility that, under aerobic conditions, creatine is actively concentrated within the cell.

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The Oxidation of Thymine by an Unidentified Bacterium. By R. D. Batt and D. D. Woods.
(*Department of Biochemistry, University of Oxford*)

Wang & Lampen (1951) have reported briefly on the oxidation of pyrimidines by a soil organism, and it may be useful to give an interim report on similar work which has been in progress here.

Two types of aerobic, rod-shaped bacteria (Gram-positive and non-sporulating) have been isolated by enrichment culture; they can utilize pyrimidines as sole source of carbon and nitrogen for growth. Cell suspensions of one organism (ST) oxidize pyrimidines even when grown on tryptic digest broth; with the other organism (SS), however, adaptive enzymes are involved.

Washed suspensions of SS derived from growth on either uracil or thymine attack both these pyrimidines but not cytosine. When grown on uracil the cells attack thymine (in phosphate buffer) at 30° with the initial accumulation of a compound which is subsequently (after a slight lag) further oxidized.

The point of inflexion in the O_2-uptake curve corresponds to an O_2 consumption of 0·5 mol. O_2/mol. thymine; no thymine is then present and only negligible amounts of NH_3, CO_2 and urea. The compound has an absorption peak at 268 mμ. and apparently a higher $\epsilon_{max.}$ value than thymine. It is unstable in acid conditions giving rise to material which does not absorb in the ultraviolet. Examination of paper chromatograms by the ultraviolet technique of Markham & Smith (1949) showed a very low R_F value with butanol-water, but a higher value (*c.* 0·3) with *iso*propanol-NH_3.

In the absence of phosphate, cell suspensions of SS may oxidize thymine in a different manner. In either water alone or in borate buffer the O_2 consumption curves are of a different type and there is less accumulation of the above absorbing compound; these differences are accompanied by changes in pH. Addition of phosphate buffer at various times during oxidation in water changes the O_2-uptake curve to the phosphate type provided thymine is still present.

Thiothymine (4-hydroxy-2-mercapto-5-methyl pyrimidine) is also oxidized in phosphate with an uptake of 0·5 mol. O_2 and with production of an ultraviolet absorbing compound. Thymine glycol (2:4:5:6-tetrahydroxy-5-methyl-5:6-dihydro pyrimidine) is not attacked. The evidence so far obtained is consistent with the view that the intermediate compound formed during thymine oxidation in phosphate buffer is uracil-5-carbinol (2:4-dihydroxy-5-hydroxymethyl pyrimidine) or a derivative.

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Further Studies on Porphobilinogen. By PHYLLIS E. BROCKMAN and C. H. GRAY. (*Department of Chemical Pathology, King's College Hospital Medical School, London, S.E.* 5)

The conversion of porphobilinogen to porphyrin and other pigments has been studied by following the changes in absorption spectra of purified porphobilinogen solutions heated at 100° at pH 5·2. Grieg, Askevold & Sveinsson (1950) have shown that maximum yields of porphyrin are obtained from porphobilinogen-containing urines on heating at this pH. After heating for 2 min. the solution became yellow in colour and showed two absorption maxima, a strong one at 477–480 mμ. and a much weaker one in the region of 350–370 mμ. On heating for a further 3 min. the absorption at these wavelengths increased, and the Ehrlich reaction was then negative. On further heating, the porphobilinogen solution became pink in colour and the absorption band at 477–480 mμ. diminished in intensity and a new maximum appeared at 408 mμ. The 408 mμ. band reached its maximum after 60 min. heating and then decreased. Throughout the whole period of heating there was a gradual increase in the general absorption. The original absorption band at 286–288 mμ. (Gray, 1951) remained unchanged except for a slight increase due to the increased general absorption, suggesting that this absorption band was due to some substance other than porphobilinogen. It now appears probable that this band was due in large part, if not entirely, to uric acid which had not been completely separated despite repeated chromatography on alumina.

The absorption maxima at 477–480 and 350–370 mμ. could be due to a dipyrrylmethene, while the 408 mμ. maximum is due to the formation of a porphyrin.

Lowry, Schmid, Hawkinson, Schwartz & Watson (1950) claimed that porphobilinogen does not give rise to porphyrin, and postulate a second precursor which can be separated from porphobilinogen. In the urines we have examined so far, there has been no evidence of the existence of this second precursor.

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The Chromatography of Steroid Benzoates. By R. V. BROOKS, W. KLYNE and E. MILLER. (*Postgraduate Medical School, London, W.*12)

Brooks, Klyne & Miller (1951), in studies on the steroids of pregnant mares' urine, reported the separation of some isomers of pregnanediol by the chromatography of their benzoates on alumina. Turner & Voitle (1951) have also reported the separation of epimeric 20-hydroxysteroids as their benzoates. The chromatography of benzoates has not been widely used by previous workers in the steroid field (but see, for example, Goldberg & Wydler, 1943; Pearlman, 1944; Barton & Miller, 1950). We have now found that many pairs of related benzoates can be separated well by chromatography.

The following pairs of compounds have been studied using alumina (Savory & Moore Ltd.) of activity II–III (Brockmann & Schodder, 1941) and light petroleum (b.p. 60–80°)—benzene mixtures of gradually increasing benzene content. (*a*) 20-Epimers: the *allo*pregnane-3β:20-diols, the pregnane-3β:20-diols and the pregn-5-ene-3β:20-diols; in each case the two epimers separated well, the 20β-isomers being eluted first. (*b*) 3-Epimers: the androstan-3-ol-17-ones, androsterone and *iso*androsterone; good separation (androsterone eluted first). (*c*) 5-Epimers: androstan-3β-ol-17-one and aetiocholan-3β-ol-17-one; partial separation (androstanolone eluted first). (*d*) 17-Epimers: the androst-5-ene-3β:17-diols (as 3-acetate 17-benzoates); good separation (17α-isomer eluted first). (*e*) 5-*allo*- and Δ^5-compounds: androstan-3β-ol-17-one and androst-5-en-3β-ol-17-one; no separation.

The following benzoates have been prepared; m.p.'s are corrected; figures in square brackets are specific rotations in $CHCl_3$ at 20–25°. Monobenzoates: aetiocholan-3β-ol-17-one, m.p. 243°, [+79°]; pregna-5:16-dien-3β-ol-20-one, m.p. 171–172°, [−9°]. Dibenzoates: pregnane-3β:20α-diol, m.p. 168–169° (cf. Pearlman, 1944), [+23°]; pregnane-3β:20β-diol, gum.

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The Metabolism of Collagen from Skin, Bone and Liver in the Normal Rat. By J. C. PERRONE* and H. G. B. SLACK.† (*National Institute for Medical Research, Mill Hill, London, N.W.*7)

In previous communications (Perrone & Slack, 1951; Neuberger, Perrone & Slack, 1951) the metabolism of collagen from normal rat tendon was studied. The results indicated a very slow rate of turnover in adult rats. These experiments have been extended to include collagen obtained from tendon, skin, bone and liver in three groups of rats of initial weight ranges 50–70, 200–240 and 350–370 g. Each rat received glycine labelled with ^{14}C in the methylene group in amounts equivalent to 10 μc./100 g. body weight. Collagen was obtained free from other proteins: (1) in tendon by a modification of the method of Bowes & Kenton (1948); (2) in skin and liver by preliminary cleaning and mincing and then extraction with 20 % (w/v) urea followed by washing with distilled water; (3) in bone by grinding, decalcification with 6 % (v/v) HNO_3, extraction by 20 % (w/v) urea followed by treatment with trypsin and subsequent washing with distilled water. All collagen samples were converted to gelatin, filtered, and the glycine present was obtained after hydrolysis as crystalline 2:4-dinitrophenyl derivative using chromatography on buffered Celite columns.

Specific activities were measured in counts/min./2 sq. cm. disk of infinite thickness. In the old rats the activities in collagen from tendon and liver were very low. Skin and bone collagens were rather more active, but all were below 100 counts/min. The levels of activity were maintained over a period of 7 weeks. In the young rats collagen glycine from all four sources gave counts of 816–944/min. at 1 week. There was some loss of activity during the first

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3 weeks, but very little further loss in activity at 8 weeks (after correcting for increases in body weight). The observed activities in all collagen samples in the young rats at 1 week were at least 10 times those from the old rats. In the rats of 200–230 g. the activities of the collagen samples were more variable, liver and bone collagen being 4–5 times as active as collagen from tendon and skin at 1 week. The interpretation of the activities found in the 200–230 g. rats is difficult since the exact ages of these rats were not known. The results indicate some metabolic activity in collagen from skin, bone, tendon and liver during the first 3 weeks in young rats, very little activity in old rats at any time, and variable metabolic activity in the 'young adult' group.

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Effect of 2:4-Dinitrophenol on Glycolysis in Mammary Gland and in Brain Tissue. By C. Terner. (*National Institute for Research in Dairying, University of Reading*)

Lactating rat mammary gland slices, incubated with glucose under anaerobic conditions, produced lactic acid at slow rates ($Q^{N_2}_{CO_2}$, +2 to +3 μl./mg. dry wt./hr.). The rate of glycolysis was accelerated by nicotinamide (0·01–0·02 M) and further increased and stabilized by pyruvate (0·001–0·05 M). In the presence of glucose, pyruvate and nicotinamide, high rates of anaerobic glycolysis were observed which remained almost linear for up to 3 hr. ($Q^{N_2}_{CO_2}$ about +10). Enzymic inactivation of cozymase, which is prevented by nicotinamide (Mann & Quastel, 1941), may explain the rapid decline of anaerobic glycolysis, previously observed by Folley & French (1949).

Under aerobic conditions only small amounts of lactic acid were formed by rat mammary tissue metabolizing glucose ($Q_{glucose}$ about −4, $Q_{lactic\ acid}$ less than +1. $Q_X = \mu$l./mg. dry wt./hr.; X determined chemically). Addition of 5×10^{-5} M-2:4-dinitrophenol (DNP) resulted in 30–40 % inhibition of $Q_{glucose}$, but at the same time an increased accumulation of lactic acid occurred in amounts equivalent to 50 % of the glucose metabolized. Mammary gland slices differ in this respect from brain cortex slices, in which $Q_{glucose}$ and $Q_{lactic\ acid}$ were increased concomitantly by even higher concentrations of DNP (2×10^{-4} M). In the presence of L-glutamate (0·01 M), the addition of both cozymase (5×10^{-5} to $1{\cdot}25 \times 10^{-4}$ M) and DNP (2×10^{-4} M) increased the rate of aerobic glucose breakdown to its anaerobic level ($Q_{glucose}$ −8·0), thus completely inhibiting the Pasteur effect. Although, in brain tissue, the increased aerobic lactic acid formation might be due to the stimulation of the rate of glucose breakdown by DNP, this cannot be the case in mammary tissue in which $Q_{lactic\ acid}$ is increased while $Q_{glucose}$ is inhibited. DNP does not inhibit $Q_{pyruvate}$ and does not increase the formation of lactic acid from pyruvate in mammary tissue (see Terner, 1951*a*).

It has been suggested (Terner, 1951*b*) that DNP causes increased operation of the oxidation-reduction step of glycolysis by inhibition of the aerobic reoxidation of reduced cozymase at the triosephosphate level. This mechanism would explain the stimulation of lactic acid formation by DNP in the absence of an increased breakdown of glucose. On this assumption the present findings would also appear to be in favour of an explanation of the Pasteur effect, at least in brain tissue, in terms of a competition between oxygen and pyruvate for the hydrogen of reduced cozymase (Ball, 1939).

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Paper Chromatography of Urinary Acids and Phenols. By R. J. Boscott (introduced by H. G. Sammons). (*Department of Anatomy, University of Birmingham*)

Phenols and acidic fractions obtained from urines by hydrolysis with hydrochloric acid or β-glucuronidase are separated into four main fractions by partition between organic solvents and aqueous phases. The extracts are separated into components using two-dimensional paper chromatography (Boscott, 1951). Aqueous alkali of high salt concentration is used as the first solvent, no organic solvent phase being present. *n*-Butyl or *n*-amyl alcohol is employed for the second solvent. Whatman no. 542 paper and the ascending technique is used.

Components are detected by (i) their fluorescence in ultraviolet light before and after spotting the paper with droplets of 90% (v/v) H_2SO_4, and (ii) their reaction with a diazonium reagent.

Results obtained by applying these procedures to human and animal urines were described.

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Urinary Components in Rats and Human Subjects with Fibrosis of the Liver. By K. Aterman and R. J. Boscott (introduced by H. G. Sammons). (*Department of Anatomy, University of Birmingham*)

In a series of experiments designed to study the role of steroid compounds in liver disease, the chromatographic technique of Boscott (1951) was applied to the separation of certain urinary components. Different chromatographic patterns were obtained in groups of rats with normal and fibrotic livers before and after the administration of steroid hormones and other compounds.

The possibility of applying the new technique to human material was investigated in a small preliminary series. In the three cases of fibrosis of the liver examined, a component was found which was not present in the urine of a few patients with other diseases and in normal urine. Small amounts of this compound were also found in one case of steatorrhoea, and in one case of toxaemia of pregnancy.

In urines from members of a family with hepatolenticular degeneration (Wilson's disease) detectable amounts of the same compound have been found even in early cases, although in one of these patients no significant liver damage was revealed by routine investigations.

Further investigations are in progress.

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Colorimetric Determination of Arylsulphatase. By D. Robinson, J. N. Smith and R. T. Williams. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W.* 2)

Arylsulphatase (phenolsulphatase) can be conveniently determined using, as substrate, potassium 2-hydroxy-4-nitrophenylsulphate (0·0025 M) made from 4-nitrophenol by the Elbs persulphate oxidation (Smith, 1951). This ethereal sulphate on hydrolysis yields 4-nitrocatechol, which on making alkaline gives a red colour ($\lambda_{max.}$ 510 mμ., $\epsilon_{max.}$ 11,300). Tissue extracts were incubated at 37° for an hour with the substrate in acetate buffer at pH 6. Protein was removed, if necessary, with phosphotungstic or trichloroacetic acid, and NaOH was then added. The red colour obtained was measured in the Spekker absorptiometer whereby 4–30 μg. of 4-nitrocatechol were readily estimated.

Defining a nitrocatechol unit of arylsulphatase activity as that amount of enzyme which will liberate 1 μg. of 4-nitrocatechol in 1 hr. at 37° at pH 6 in acetate buffer, the relative activities of 1 g. of various materials were found to be as follows: takadiastase, 850; rat tissues, spleen, 300–400; lung, 100–200; liver, 25–70; heart, 70–80; kidney, 60–110; intestine, 30–50. We have also examined the 'saliva' of locusts (*Locusta migratoria migratorioides*) and found it to contain 800–1000 units/ml.

of arylsulphatase activity. None was found in human saliva.

The action of takadiastase on two ethereal sulphates yielding nitroquinol on hydrolysis has also been studied. Nitroquinol in alkali gives a purple but unstable colour ($\lambda_{max.}$ 540 mμ., $\epsilon_{max.}$ 3850). 4-Hydroxy-2-nitrophenylsulphate is readily hydrolysed by takadiastase, but its isomer 4-hydroxy-3-nitrophenylsulphate is not.

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Sucrose Breakdown by Enzyme Preparations from Moulds. By F. J. Bealing and J. S. D. Bacon. (*Department of Biochemistry, University of Sheffield*)

The invertase of *Aspergillus oryzae* has been regarded as a 'glucosaccharase', because it is inhibited by free glucose and fails to attack raffinose (cf. Neuberg & Mandl, 1950; Gottschalk, 1950). An investigation of the action of various mould enzyme preparations on sucrose has shown that this view is probably incorrect.

In a typical experiment a dialysed extract of *A. oryzae*, obtained by autolysis under toluene, was incubated with 7·5% sucrose in 0·01 M-acetate buffer (pH 5·0), and the reaction mixture analysed by quantitative paper partition chromatography (Bacon & Edelman, 1951). After 3 hr. at least 80% of the sucrose had disappeared, but only 7·5% of the fructose and 42·5% of the glucose expected from simple hydrolysis was present in the free state; the remainder appeared in the form of non-reducing substances with R_F values less than that of sucrose. These compounds were hydrolysed after incubation for several days, giving only fructose and glucose; they were not identical with those produced by yeast invertase (Blanchard & Albon, 1950; Bacon & Edelman, 1950).

The rate of formation of free fructose was not influenced by variations in the sucrose concentration, nor by the addition of glucose. The rate of incorporation of fructose in oligosaccharides other than sucrose was trebled when the initial sucrose concentration was raised from 8 to 40%; in the presence of glucose it was substantially reduced.

The above results may be explained by assuming that the enzyme preparation transfers fructose residues from sucrose to any carbohydrate molecules present in the reaction mixture, and also to water; and that the products of these transfer reactions, with the exception of free fructose, then act as substrates for further transfer reactions, the reaction ending when all fructose residues have been transferred to water. This hypothesis is supported by the observation that when sucrose is incubated with mould enzyme preparations in the presence of various alcohols, fructose-containing substances are formed having R_F values greater than that of free fructose; a similar observation has been made with yeast invertase. That a single enzyme is responsible for both hydrolytic and transferring activities is suggested by the fact that preparations from different species, from mycelia of different ages, and from crushed spores (Hughes, 1951), give rise to quantitatively similar mixtures of free sugars and oligosaccharides during their action on sucrose solutions of the same concentration.

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The Metabolism of ^{14}C-labelled Bicarbonate in the Cat. By H. L. Kornberg, R. E. Davies and D. R. Wood. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry; and Department of Pharmacology and Therapeutics, University of Sheffield*)

In the course of studies on the metabolism of labelled urea in the cat (Davies & Kornberg, 1950) it was necessary to obtain information on the rate of excretion of $^{14}CO_2$, and on the rate of incorporation of ^{14}C into urea, after injection of ^{14}C-labelled bicarbonate. Much work on the former topic has been published in recent years, but this was usually carried out on mice (Skipper, White & Bryan, 1949;

Skipper, Nolan & Simpson, 1950) or rats (Armstrong & Schubert, 1949; Armstrong & Zbarsky, 1949; Gould, Sinex, Rosenberg, Solomon & Hastings, 1949; Greenberg & Winnick, 1949; Schubert & Armstrong, 1949), in which the $NaH^{14}CO_3$ was injected intraperitoneally. However, this method of administration of the isotope, and the differences in body size between mice and rats, and cats, made it impossible to apply the earlier results to our studies.

Cats were anaesthetized with ether and chloralose (75 mg./kg. body wt.) and connected through a valve and cannula inserted in the trachea to a special respiration circuit by means of which the cat freely inspired CO_2-free air, the expired CO_2 being continuously collected in 15% NaOH for definite lengths of time. The ureters were tied and $NaH^{14}CO_3$ ($5–7 \times 10^5$ counts/min.) injected intravenously. The rate of expiration of $^{14}CO_2$ is initially very rapid, and can be expressed as an exponential equation with three components, the curves obtained in our experiments corresponding to the function:

$$P = 28{\cdot}4e^{-0{\cdot}243t} + 41{\cdot}5e^{-0{\cdot}0234t} + 30{\cdot}2e^{-0{\cdot}00119t},$$

where P is the percentage of injected ^{14}C left in the cat at time t. In the first 30 min. 50% of the injected isotope is expired, by which time the blood bicarbonate had lost all but 5%. The total excretion over 5 hr. was 80–82%. The rate of incorporation of ^{14}C into urea follows closely the rate of expiration of $^{14}CO_2$. After 5 hr. the urea carbon contained 3·1% of the injected ^{14}C; over 50% of this was incorporated in the first 40 min. Our data show that within the experimental error (±2%) all the urea carbon is derived from CO_2 in equilibrium with blood bicarbonate. This confirms the results obtained *in vitro* by Grisolia & Cohen (1948) and *in vivo* by Mackenzie & du Vigneaud (1948).

The results show that there are several 'CO_2 cycles' operating, since the injected $NaH^{14}CO_3$ equilibrated rapidly with the total blood bicarbonate and at varying rates with other carbon pools including the bicarbonate in the tissues. After 5 hr. 10–12% of the ^{14}C injected had been incorporated into the tissues in forms which exchange relatively slowly with the bicarbonate of the blood. Most of this was present in bone.

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Sources of Error in the Assay of β-Glucuronidase in Animal Tissues. By P. G. Walker and G. A. Levvy. (*Rowett Research Institute, Bucksburn, Aberdeenshire*)

Considerable attention has been paid to comparative measurements of the glucuronidase activity of different tissues, or of a single tissue in different normal and pathological states. Most methods of assay make use of the supernatant obtained by adding acetic acid or citrate or acetate buffer to the water homogenate, and incubating for varying periods before centrifuging. The introduction of phenolphthalein glucuronide as substrate (Talalay, Fishman & Huggins, 1946) has made possible the use of the untreated water homogenate.

In work already reported in part (Kerr, Levvy & Walker, 1950; Kerr & Levvy, 1951), it was found that after homogenizing mouse liver in water some of the glucuronidase activity was associated with subcellular particles. Acidification led to agglutination and rapid sedimentation of the particles. In dilute preparations, the overall homogenate activity was equal to the sum of the soluble and insoluble fractions. The insoluble enzyme gradually went into solution on incubation in acetate buffer, but not in citrate buffer, nor in preparations acidified with acetic acid. Various other measures have been found to bring the insoluble enzyme into solution, for example, addition of the surface-active agent Triton X-100 or mechanical disintegration in the Waring blender.

From the study of homogenates in isotonic media it has been concluded that by far the greater part of the glucuronidase activity in mouse liver is originally associated with subcellular particles. Such homogenates did not show their full glucuroni-

dase activity on direct assay, and required preliminary activation for reliable measurement. All comparable measurements have, however, been in agreement in confirming the observation of Levvy, Kerr & Campbell (1948) that in the mouse the glucuronidase activity is higher in infant liver and in liver regenerating after partial hepatectomy than in the normal adult tissue.

Preliminary results in a similar investigation of rat liver suggest that this tissue contains both soluble and insoluble glucuronidase, but otherwise behaves very differently from mouse liver in the enzyme assay. Water homogenates of rat liver did not initially display their full activity (as realized by adding Triton X-100) in acetate buffer, and the discrepancy was very much greater in infants than in adults. Full activity was displayed by adult, but not by infant liver, after preliminary incubation in the buffer for 2 hr. In citrate buffer, true values were always obtained for adult liver, and with infant liver the discrepancies were less marked than in acetate. On our present data, any opinion as to the relationship between the glucuronidase activity of rat liver and its state of proliferation would be premature.

This work has obvious bearing on the comparative measurements of other enzymes.

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An Amylolytic Degradation of Rabbit-liver Glycogen. By D. J. Bell and D. J. Manners. (*School of Biochemistry, University of Cambridge*)

A large batch of glycogen (unit chain length by both periodate and methylation methods, 12; $[\alpha]_D$ in water +198°, and mol. wt. by sedimentation-diffusion (Bell, Gutfreund, Cecil & Ogston, 1947), approx. $4{\cdot}5 \times 10^6$) was subjected to stepwise degradation by the β- and α-amylases of barley. At pH 4·6, β-amylase (free from α) gave a high mol. wt. dextrin (L.D. 1) (74 %) and maltose (26 %). L.D. 1 gave a yellow-brown colour with iodine, its solution in water was opalescent and had $[\alpha]_D$ +194°. In N-NaOH $[\alpha]_D$ was +159° while the parent glycogen had $[\alpha]_D$ +169°. The unit chain length by periodate was 9. Mol. wt. by light-scattering (through the co-operation of Dr B. S. Harrap of the Department of Colloid Science) was $3{\cdot}9 \times 10^6$ compared with the value found for the parent glycogen, namely $5{\cdot}9 \times 10^6$. The 'reducing powers' (as maltose) of L.D. 1 and the glycogen were respectively, (*a*) by copper method, nil and nil, (*b*) by hypoiodite, 3·4 % and 2·7 %. Total N (Kjeldahl) was 0·05 % for the glycogen and 0·12 % for L.D. 1.

L.D. 1 treated with α-amylase (free from β-) at pH 5·4 gave a low mol. wt. dextrin, L.D. 2 (76 %) and 24 % of reducing sugars (maltose and a little glucose). L.D. 2 gave no colour with iodine; the aqueous solution was clear and $[\alpha]_D$ was +186°. Reducing power (as maltose) was (*a*) copper method, 12·3 %, (*b*) hypoiodite 14·0 %, 'degree of polymerization' (calc. from Cu reduction) was approx. 17 and from this an approximate mol. wt. of 2800 can be deduced. Total N (Kjeldahl) was 0·22 %.

L.D. 1 treated simultaneously with both enzymes at pH 5·4 gave 74 % conversion to a heterogeneous mixed dextrin (L.D. 3) along with maltose and some glucose. L.D. 3 gave no colour with iodine, gave a clear solution in water and had $[\alpha]_D$ +179°. Reducing power as maltose was (*a*) by copper method, 18·0 %, (*b*) by hypoiodite, 19·3 %. 'Degree of polymerization' (calc. from Cu reduction) was approx. 12 and from this an approximate mol. wt. of 2000 could be deduced. Total N (Kjeldahl) was 0·22 %.

In all digests the reaction was stopped when a constant copper reduction had been attained. This was measured by a slight modification of Somogyi's (1937) method. The dextrins were separated by ethanolic precipitation.

L.D. 3 was separated by dialysis into two fractions differing markedly in $[\alpha]_D$ and 'degree of polymerization', namely, approx. 6 and approx. 16, respectively. L.D. 3 was shown to undergo further degradation by salivary amylase.

Our results on β-amylolysis of a 12-radical glycogen are of interest when compared with the findings of Halsall, Hirst, Hough & Jones (1949) in the case of an 18-radical specimen. Both residual dextrins (i.e. L.D. 1) must have chain lengths of approximately nine to ten radicals and the glycogens therefore differ presumably with respect to the external chains. Halsall *et al.* used wheat and crystalline sweet-potato β-amylases. On the other hand, Meyer & Fuld (1941) found 50 % degradation

of an 11-radical glycogen from mussels; it would seem that this glycogen may differ from rabbit-liver polysaccharide with respect to the location of the point of branching.

The loss of an average of three glucose radicals per unit chain during β-amylolysis, together with the selective fission of certain interior chains by the α-enzyme, would be in accord with the view put forward by Meyer & Fuld (1941) that the glycogen molecule is not only highly branched but that the branches are irregular.

We wish to acknowledge valuable advice and help given by Dr C. S. Hanes, F.R.S., and Mr E. T. Whitmore in connexion with the enzymic work in the initial stages.

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The Amino Groups of Conalbumin, Ovomucoid and Avidin. By H. Fraenkel-Conrat (Rockefeller Foundation Fellow) and R. R. Porter (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

There occur in egg white four singular proteins of markedly specific biological activities. It appeared of interest to apply to these the techniques of Sanger (1945) and Porter & Sanger (1948) for the characterization and determination of amino groups. The proteins investigated were conalbumin, ovomucoid and avidin, lysozyme having been studied elsewhere (Green & Schroeder, 1951).

Conalbumin presented no unusual problems. It appeared to contain a single terminal amino group per mole, which was identified as alanine (1·0, average of four experiments, range 0·9–1·1). Since a greater number of peptide chains had been postulated on an analytical basis (Lewis, Snell, Hirschmann & Fraenkel-Conrat, 1950), unsuccessful attempts were made to reveal more end groups after various types of denaturation (found 1·2/mole, three experiments). The amount of ε-dinitrophenyl-lysine found was in good agreement with the microbiological assays for this amino acid (Lewis *et al.* 1950) (62 and 60 residues/mole).

The DNP-ovomucoid was soluble in water and aqueous ethanol, so that it had to be isolated by dialysis. The breakdown of dinitrophenyl-alanine and ε-dinitrophenyl-lysine when boiled for 16 hr. with 20 % HCl in the presence of ovomucoid was 40 and 30 %, respectively, higher than losses previously reported with other proteins (Porter & Sanger, 1948). This is presumably due to the high carbohydrate content of ovomucoid. When these corrections for losses are applied, one terminal alanine per mole was found (0·96, six analyses ranging from 0·8–1·2); the corrected lysine value is somewhat higher than the lysine found microbiologically (13·7 and 12 residues/mole).

With various avidin preparations, both nucleoprotein and nucleic acid free, rather erratic results were obtained. It appears, however, that all the lysine ε amino groups (26) react, and that there are three N-terminal alanine residues per mole.

Both with ovomucoid and with avidin, there was often a small amount of another slow-moving yellow component resembling glycine detectable on silica gel columns developed with chloroform, amounting always to less than half an equivalent per mole. It appears more likely that it represents an artifact, possibly characteristic of glycoproteins, rather than a genuine end group of a contaminating protein.

No evidence was found for reaction of fluoronitrobenzene with components of the nucleic acid of avidin, or with pure thymonucleic acid.

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The Absorption of Inorganic Phosphate from the Rumen of the Sheep. By R. SCARISBRICK and T. K. EWER. (*Department of Animal Pathology, University of Cambridge*)

The saliva of ruminants is voluminous and very rich in inorganic phosphate; this causes a large movement of phosphorus from the blood into the rumen, where the concentration is kept high. The present experiments were undertaken with sheep to see whether inorganic phosphate is absorbed from the rumen. The ruminal vein and the carotid artery of sheep were exposed under nembutal or chloral anaesthesia and the inorganic phosphate content of the blood in these vessels compared.

An injection of inorganic phosphate containing ^{32}P was made into the rumen of the first sheep: within 10 min. the isotope appeared in the blood in the ruminal vein and to a less extent in the blood in the carotid artery and was also present in the saliva. Subsequent samples of blood and saliva contained less isotope.

Phosphorus (2·8 g., as a mixture of sodium dihydrogen phosphate and disodium hydrogen phosphate at pH 6·4) was injected into the rumen of the second sheep. Five minutes after the injection the inorganic phosphate content of the blood in the ruminal vein had risen from 6·3 to 24 mg. P/100 ml. and in the carotid artery from 5·8 to 17 mg. P/100 ml.

These results indicated that absorption of inorganic phosphate from the rumen is possible and further determinations were carried out to assess the extent of such absorption when the contents of the rumen are not disturbed. Experiments on four more sheep showed that under these circumstances the amount of absorption was very variable, varying considerably in the same animal from time to time. The mean of all determinations of the arterio-venous difference of inorganic phosphate was only 0·24 mg. P/100 ml. The extent of the variability is shown by the high value of the standard deviation of the a.v. differences, 0·93 mg. P/100 ml.; on several occasions inorganic phosphate appeared to pass from the blood to the rumen. The net absorption of inorganic phosphate from the rumen over a long period of time, therefore, seems to be at most only small in amount, but at any particular instant there may be a substantial movement of inorganic phosphate into or out of the blood traversing the ruminal wall.

Acetate Metabolism of *Aerobacter aerogenes*. By S. DAGLEY, G. A. MORRISON (*Department of Biochemistry, University of Leeds*) and E. A. DAWES (*Department of Biochemistry, University of Glasgow*)

Aerobacter aerogenes was trained to grow on aerated synthetic media utilizing ammonia as nitrogen source with acetate, succinate, fumarate or malate as sole sources of carbon, and rates of production of pyruvate, as determined by the method of Friedemann & Haugen (1943), were measured for aerated washed suspensions of the trained cells. Considerable training was necessary before acetate was utilized readily for growth: training to metabolize other substrates (e.g. glucose, malate, fumarate, succinate) did not confer adaptation to acetate (Baskett & Hinshelwood, 1950); and production of pyruvate by cells trained to utilize other substrates was feeble when aerated in acetate-phosphate buffer. When trained to grow on acetate, however, cells were not only adapted to succinate, fumarate and malate, but pyruvate was produced readily from acetate as well as from the dicarboxylic acids.

From our results it appears that the pathway by which acetate-grown cells produce the pyruvate for growth requirements may be the 'dicarboxylic acid cycle' suggested by Barron, Ardao & Hearon (1950) in their studies with *Corynebacterium creatinovorans* and supported by recent work with *Escherichia coli* using radioactive carbon (Ajl & Kamen, 1951).

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