PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **344th Meeting** *of the Biochemical Society was held in the Department of Physiology, Bedford College, Inner Circle, Regent's Park, N.W.* 1, *on* 22 *October* 1955, *at* 2.00 *p.m. when the following papers were read:*

COMMUNICATIONS

The Effect of Adenosine on the Movement of Sodium between Erythrocytes and the Suspension Medium.

By E. J. Harris and T. A. J. Prankerd. (*Department of Biophysics, University College, and Medical Unit, University College Hospital Medical School, London*)

The rates of entry and extrusion of the major part of the Na of red cells depend upon the condition of the cells. Storage *in vitro* overnight leads to diminution of both rates (E. J. Harris & Prankerd, 1953). Since Prankerd & Altman (1954) have shown that the addition of adenosine to a suspension will maintain or even lead to restoration of the cell organic phosphates it seemed likely that Na movement might also be affected by this substance.

When added to a suspension of fresh cells in a diluted plasma containing labelled Na, 0·005 M adenosine in some experiments had no effect, and in some caused an increase in cell Na, without changing the rate of output of the main part of the cell Na (the cell Na does not behave homogeneously). For example, with glucose the inward rate constant was 0·065 hr.$^{-1}$, and outward rate constant 0·35 hr.$^{-1}$, with adenosine the figures were 0·092 and 0·36 hr.$^{-1}$ respectively. The effect on cells which had been kept *in vitro* was upon both inward and outward rate constants. For example, using the same batch of cells: with glucose—inward 0·031, outward 0·176 hr.$^{-1}$; with adenosine—inward 0·126, outward 0·374 hr.$^{-1}$. In another experiment, using a different batch of cells, the figures were: with glucose—inward 0·025, outward 0·072 hr.$^{-1}$; with adenosine—inward 0·034, outward 0·125 hr.$^{-1}$.

The adenosine added to the suspension is degraded to adenine, and this occurs whether or not additional glucose is added. Cells incubated with ouabain (10 μg./ml.) gain Na and lose K (as found for cardiac glycosides and aglycones by Schatzmann, 1953), but the presence of adenosine protects the cells from this effect. Thus there is evidence that ion movements can be potentiated either by glucose (J. E. Harris, 1941) or by adenosine, and that the latter substance better maintains the outward Na movement of stored cells.

REFERENCES

Harris, E. J. & Prankerd, T. A. J. (1953). *J. Physiol.* **121**, 470.
Harris, J. E. (1941). *J. biol. Chem.* **141**, 579.
Prankerd, T. A. J. & Altman, K. I. (1954). *Biochem. J.* **58**, 622.
Schatzmann, H. J. (1953). *Helv. physiol. acta*, **11**, 346.

The Interaction of Proteins and Aminostilbenes.

By D. F. Cheesman. (*Department of Physiology, Bedford College, University of London*)

It has been reported (Cheesman, 1954) that those compounds related to 4-aminostilbene which have a nucleotoxic action (Haddow, Harris, Kon & Roe, 1948) produce an apparent cross-linking effect on unimolecular films of the protein globin, in that they lower the gelation pressure of the films and greatly increase the mechanical strength of protein fibres drawn from the surface.

Experiments have been made to elucidate the mechanism of this action, which was scarcely to be expected with compounds containing a single polar group. Films were spread on the Langmuir trough from solutions in 80% (v/v) *iso*propanol of beef globin hydrochloride (0·4 mg./ml.) with and without admixed 4-dimethylamino-2′-methyl-*trans*-stilbene (DAMS, 0·1 mg./ml.). The substrate was 0·02 M-Na_2HPO_4-KH_2PO_4 buffer. The gelation pressure was determined by observation of the movements of talc particles floating in the surface.

It has been established that the reduction in gelation pressure (Δp) caused by DAMS is highly dependent upon pH, showing a pronounced maximum at pH 6·7, about which the Δp/pH curve is symmetrical. This maximum corresponds approximately to the isoelectric point of native globin. When the globin solution is boiled or aged before spreading, the maximum is flattened and displaced to pH 6·9. A similar displacement of the isoelectric point is to be expected (cf. Putnam, 1953). The film gelation points of zein and gliadin, proteins without free carboxyl groups, are unaffected by DAMS.

These observations suggest that an equivalence of ionized basic and carboxyl groups in the protein

favours the cross-linking action, and hence that the active aminostilbene molecules are strongly polarized by interaction with suitably disposed ion pairs on the protein surface. The cross-linking would then involve the establishment of electrovalent bonds between the aminostilbene N atom and a protein carboxyl group, and between, say, the 4′-C atom of the aminostilbene and a protein basic group.

Such a polarization, which would involve an increased contribution of quinonoid structures to the molecule, might be expected to cause a bathochromic shift in the ultraviolet absorption spectrum of the aminostilbene. Differential spectrometry on mixed solutions of native beef globin (0·2 mg./ml.) and DAMS (0·5 μg./ml.) showed this shift to be very marked (5–15 mμ.) in the pH range 4–9. It is accompanied in the central part of this range by a maximum or an inflexion at about 345 mμ., which is absent or less pronounced in the absence of protein, although the principal maximum appears near this wavelength for solutions of DAMS in ethanol (Haddow *et al.* 1948; Beale & Roe, 1952).

REFERENCES

Beale, R. N. & Roe, E. M. F. (1952). *J. Amer. chem. Soc.* **74**, 2302.

Cheesman, D. F. (1954). *Biochem. J.* **56**, xlii.

Haddow, A., Harris, R. J. C., Kon, G. A. R. & Roe, E. M. F. (1948). *Phil. Trans.* A, **241**, 147.

Putnam, F. W. (1953). *The Proteins.* Ed. Neurath, H. & Bailey, K., vol. I, p. 850. New York: Academic Press.

The Enzymic Interconversion of Pyridoxal Phosphate and Pyridoxamine Phosphate by Aqueous Extracts of *Escherichia coli*. By R. B. Beechey and F. C. Happold. (*Department of Biochemistry, University of Leeds*)

In unpublished work the authors have shown that, over a wide range of conditions, pyridoxal phosphate is the only member of the vitamin B_6 group that will activate apotryptophanase. However, on incubation of pyridoxamine phosphate with aqueous extracts of apotryptophanase at pH 8·2, activation of the apotryptophanase occurs. In the presence of Mg^{2+}, optimal concentration 2×10^{-3} M, this effect was greatly enhanced. Other divalent metal ions, i.e. Mn^{2+}, Co^{2+}, Zn^{2+}, Fe^{2+} and Cu^{2+}, showed no activating effect. These results suggested the conversion of pyridoxamine phosphate to pyridoxal phosphate.

Peaks in the ultraviolet absorption spectrum of pyridoxal phosphate and pyridoxamine phosphate, at pH 8·2, occur at 390 and 320 mμ. respectively. The enzymic conversion of pyridoxamine phosphate to pyridoxal phosphate was confirmed by following the decrease in optical density at 320 mμ. and the simultaneous increase at 390 mμ. which occurred when aqueous *Escherichia coli* extracts were incubated with pyridoxamine phosphate at 37°, pH 8·2. Similarly, the conversion of pyridoxal phosphate to pyridoxamine phosphate was demonstrated by following the decrease in optical density at 390 mμ. and the simultaneous increase at 320 mμ. Mg^{2+} appears to accelerate only the conversion of pyridoxamine phosphate to pyridoxal phosphate.

Incorporation of [^{14}C] Amino Acids into Rat Liver and Hepatoma Proteins *in vitro*. By P. N. Campbell and Nancy E. Stone. (*Courtauld Institute of Biochemistry, Middlesex Hospital, London, W.* 1)

An attempt has been made to follow the synthesis of a specific protein, serum albumin, in slices of rat liver and hepatoma, and to compare the rate of its synthesis with that of the other proteins in the two tissues.

The method of Peters & Anfinsen (1950) for following the synthesis of albumin in chick liver slices has been adapted. Rat serum albumin was purified by ammonium sulphate precipitation, followed by zone electrophoresis on cellulose columns (Porath, 1954). Antiserum was obtained by injecting an alum precipitate of the albumin into rabbits. Hepatomas were produced in rats by including *p*-dimethylaminoazobenzene in the diet (Kline, Miller, Rusch & Baumann, 1946).

Slices, obtained from the non-cancerous lobes of livers bearing small hepatomas and from the hepatomas, were incubated aerobically in a medium containing [^{14}C]glycine for periods up to 4 hr. After incubation the slices were removed from the medium and homogenized in buffer. A clear supernatant was obtained from the homogenate by centrifugation. Albumin was precipitated from the incubation medium and from the supernatant by addition of antiserum. The total protein in medium and supernatant was precipitated with trichloroacetic acid. Comparison of the radioactivity of these protein fractions showed that whereas the radioactivity of the trichloroacetic acid precipitate from hepatoma

was much greater than that from liver, the hepatoma albumin was slightly less active than the liver albumin.

These results suggest that the higher rate of incorporation of radioactive amino acids into the soluble proteins of hepatoma compared with liver cannot be explained by differences in the sizes of the free amino acid pools in the two tissues. Thus if incorporation of radioactive amino acids can be interpreted as a measure of protein synthesis, it may be concluded that in general the synthesis of proteins in hepatoma slices is more rapid than that in liver slices.

When the slices were incubated with [^{14}C]glucose instead of [^{14}C]glycine both the albumin and the trichloroacetic acid precipitated protein of the hepatoma were more radioactive than those from liver. This difference can be accounted for on the basis that the dilution of [^{14}C]glucose by inactive intracellular glucose is greater in the case of liver slices than it is in hepatoma slices.

REFERENCES

Kline, B. E., Miller, J. A., Rusch, H. P. & Baumann, C. A. (1946). *Cancer Res.* **6**, 1.

Peters, J. jun. & Anfinsen, C. B. (1950). *J. biol. Chem.* **186**, 805.

Porath, J. (1954). *Acta chem. scand.* **8**, 1813.

Effect of 2:4:6-Triethyleneimino-1:3:5-triazine on Carbohydrate Metabolism in Ascites Tumour Cells. By I. M. Roitt. (*Courtauld Institute of Biochemistry, Middlesex Hospital, London, W.* 1)

The potent growth-inhibitory properties of the ethyleneimines suggested that a study of the interaction of these compounds—in particular 2:4:6-triethyleneimino-1:3:5-triazine (TEM)—with carbohydrate metabolism in normal and malignant mammalian cells would be of interest.

Anaerobic glycolysis in Krebs mouse ascites tumour cells was completely inhibited by 0·001 M TEM. Higher concentrations were required to inhibit anaerobic glycolysis in slices of rat brain cortex, spleen and kidney cortex, but the sensitivity of liver and hepatoma to the inhibitor was of the same order as that of the ascites tumour. Respiration in ascites tumour cells, brain and kidney slices was also affected by TEM. A marked delay in the onset of these inhibitions was prominent, and was not due to slow penetration of the inhibitor into the cells or to the occurrence of a slow reaction of TEM with the Krebs Ringer medium to yield an inhibitory substance.

Since TEM reacts with the sulphydryl groups of cysteine, glutathione and β-mercaptoethylamine, its effect on the activity of the 'SH-enzymes' hexokinase, triosephosphate dehydrogenase and phosphofructokinase was studied. Only the latter was strongly inhibited. However, analysis of phosphorylated intermediates (LePage, 1949) in ascites tumour cells which had been inhibited by TEM under anaerobic conditions revealed a greater accumulation of fructose diphosphate than of the monophosphate; triosephosphate concentration was higher in the inhibited cells than in the controls but phosphoglyceric acid concentration was unaltered. Although it was shown that extracts of the TEM-inhibited cells contained active triosephosphate and lactic acid dehydrogenases, addition of pyruvate to the inhibited cells failed to restore glycolysis, suggesting that the concentration of DPN was inadequate.

Concurrent with the onset of inhibition, the DPN concentration fell to less than 20% of the control value and no DPN could be detected in the surrounding medium. This rate of fall was much higher than that observed when glycolysis was arrested by anaerobic incubation of the cells at 38° in the absence of glucose. There was no direct reaction between DPN and TEM. Nicotinamide afforded protection against the inhibitory action of TEM and could also reverse the inhibition. The low concentration of DPN appears to be the cause rather than the consequence of the inhibition by TEM, the glycolytic enzymes themselves remaining seemingly unaffected.

REFERENCE

LePage, G. A. (1949). In Umbreit, W. *Manometric Techniques and Tissue Metabolism*, p. 185.

The Effects of Glutathione on Potassium Toxicity in Rats. By D. F. Cole. (*Department of Medicine, University of Durham, Medical School, Newcastle-on-Tyne* 1)

Subcutaneously injected glutathione increases the resistance of both intact and adrenalectomized mice to potassium poisoning (Zwemer, Vollmer & Carey, 1951; Martorano, Zwemer & Vollmer, 1953). It seemed possible that the protection against potassium poisoning afforded by glutathione and that which is caused by adrenal cortical steroids (Zwemer & Truszkowski, 1936; Truszkowski & Duszynska, 1940) might show common features. We have therefore studied renal excretion

of potassium, and potassium concentrations in skeletal and cardiac muscle after a single injection of potassium chloride, given to rats either with or without previous glutathione treatment. The effects of previous adrenalectomy and of feeding high- or low-potassium diets were also investigated.

We confirmed the protective action of glutathione, but did not observe any increase of renal potassium excretion nor did its action depend on preventing any increase of skeletal or cardiac muscle potassium. In these respects glutathione protection differs fundamentally from the action of deoxycorticosterone or adrenal cortical extract (Ferrebee, Parker, Carnes, Gerity, Atchley & Loeb, 1941; Harkness, Muntwyler, Mautz & Mellors, 1942). Although intact rats given high-potassium diets showed values for tissue potassium as high, or higher than, adrenalectomized rats, signs of potassium poisoning were evident only in the adrenalectomized series. This suggests that the appearance of toxic effects is not entirely dependent on tissue concentrations of potassium. It is also suggested that glutathione protection depends on alterations of tissue metabolism dissimilar to those caused by mineralo-corticoids. Recently, Booker, Tureman, Dacosta, Poulson & Mitchell (1955) also concluded that glutathione and cortisone differ in the manner in which they protect mice against cold-stress.

This work was carried out during the tenure of an I.C.I. Research Fellowship.

REFERENCES

Booker, W. M., Tureman, J. R., Dacosta, F. M., Poulson, J. & Mitchell, S. Q. (1955). *Amer. J. Physiol.* **181**, 371.

Ferrebee, J. W., Parker, D., Carnes, W. H., Gerity, M. K., Atchley, D. W. & Loeb, R. F. (1941). *Amer. J. Physiol.* **135**, 230.

Harkness, D. M., Muntwyler, E., Mautz, F. R. & Mellors, R. C. (1942). *J. Lab. clin. Med.* **28**, 307.

Martorano, J. J., Zwemer, R. L. & Vollmer, E. P. (1953). *Amer. J. Physiol.* **173**, 1.

Truszkowski, R. & Duszynska, J. (1940). *Endocrinology*, **27**, 117.

Zwemer, R. L. & Truszkowski, R. (1936). *Science*, **83**, 558.

Zwemer, R. L., Vollmer, E. P. & Carey, M. M. (1951). *Amer. J. Physiol.* **164**, 766.

A Comparison of the Dry Weights of Isolated Cell Nuclei, as determined by Chemical and by Interferometric Methods. By A. J. HALE and E. R. M. KAY (introduced by J. N. DAVIDSON). (*Institute of Physiology and Department of Biochemistry, The University, Glasgow*)

The amount of certain materials present in isolated cell nuclei depends upon the procedure used to isolate these nuclei (Dounce, 1955). It is not known whether the mean dry weight of millions of nuclei, as determined by bulk chemical methods, is the same as the mean dry weight of tens, as determined by interferometric microscopy, or whether the method of isolation affects the result.

The nuclei of calf's thymus were isolated (*a*) by the citric acid method (CN) of Mirsky & Pollister (1946), (*b*) by the non-aqueous method (NAN) of Kay, Smellie, Humphrey & Davidson (1955), and (*c*) by the sucrose-calcium chloride method (SN) of Schneider & Peterman (1950).

The chemical determination made use of the method described by Smellie, Humphrey, Kay & Davidson (1955) for the estimation of deoxyribonucleic acid phosphorus (DNA-P) and ribonucleic acid phosphorus (RNA-P). The mean dry weight of the CN and SN were estimated by counting the nuclei in an aliquot of each using a haemocytometer, weighing the dry residue of a similar aliquot and thereby calculating the dry weight per nucleus. The mean dry weight of NAN was estimated by measuring the DNA-P content of a known weight of NAN and calculating the number of nuclei present by dividing the total DNA-P by the mean DNA-P per nucleus obtained from the CN, and then dividing the known weight of the sample by the number of nuclei present.

Interferometric determination of the dry weight of individual nuclei was made by the trace displacement method described by Davies, Wilkins, Chayen & LaCour (1954). Approximately thirty-five nuclei were examined in each sample by this method.

The mean dry weight of the CN was 19·1 and 19·8 pg. as determined by chemical and interferometric methods respectively. The corresponding values for SN were 23·0 and 21·1 pg. and those for NAN were 33·0 and 35·2 pg. The interferometric method showed that there was a considerable scatter about the mean values in all samples.

We thank the Advisory Committee on Medical Research in Scotland for a grant and the National Cancer Institute of Canada for a Research Fellowship (E. R. M. K.).

REFERENCES

Davies, H. G., Wilkins, M. H. F., Chayen, J. & LaCourr, L. F. (1954). *Quart. J. micr. Sci.* **95**, 271.

Dounce, A. L. (1955). *The Nucleic Acids*, vol. II, chap. 3. (Ed. Chargraff, E. & Davidson, J. N.) New York: Academic Press.

Kay, E. R. M., Smellie, R. M. S., Humphrey, G. F. & Davidson, J. N. (1955). *Biochem. J.* (in the Press).

Mirsky, A. E. & Pollister, A. W. (1946). *J. gen. Physiol.* **30**, 117.

Schneider, R. M. & Peterman, M. L. (1950). *Cancer Res.* **30**, 751.

Smellie, R. M. S., Humphrey, G. F., Kay, E. R. M. & Davidson, J. N. (1955). *Biochem. J.* **60**, 177.

Uptake of B_{12}-Vitamins in *Ochromonas malhamensis*. By J. E. Ford, Margaret E. Gregory and E. S. Holdsworth. (*The National Institute for Research in Dairying, University of Reading*)

The protozoan *Ochromonas malhamensis*, like certain higher animals, is narrowly selective in its requirement for 'vitamin B_{12}'. Of the naturally occurring B_{12} vitamins, only cobalamin and vitamin B_{12III} are active (cf. Coates & Ford, 1955), and under conditions specified for the assay of vitamin B_{12} with this micro-organism the other vitamin B_{12}-like compounds do not interfere.

In a culture of *Ochromonas* grown with limiting cyanocobalamin, added cyanocobalamin, vitamin B_{12III}, pseudovitamin B_{12} or factor A were taken up by the cells in about the same amount. Factor B was taken up very little, if at all.

Above a certain critical concentration in the culture liquor, the rate of uptake of added cyanocobalamin increased sharply. Furthermore, the uptake of cyanocobalamin added at a concentration (0·0005 μg./ml. of culture) below this critical level was progressively increased by the simultaneous addition of pseudovitamin B_{12} or factor A in concentrations between 0·001 and 0·006 μg./ml. At higher concentrations the uptake of cyanocobalamin was increasingly inhibited. Factor B was without effect.

On measuring the rate of the growth response to a limiting concentration of cyanocobalamin, we found that at low levels ($<0{\cdot}02$ μg./ml. culture medium) pseudovitamin B_{12} and factor A were slightly stimulatory, but at higher concentrations they retarded growth. Again, factor B was without effect.

In more detailed experiments with pseudovitamin B_{12} we established that, except at relatively low concentration, the analogue inhibits competitively the growth response to cyanocobalamin.

Within a wide range of concentration the influence of pseudovitamin B_{12} and factor A was manifest only in the *rate* of growth. The peak cell population densities attained in the cultures were determined by the limiting concentrations of cyanocobalamin supplied in the medium.

These findings may be linked with our observation that extracts of *Ochromonas* cells contain a substance which combines with cyanocobalamin, factor A or pseudovitamin B_{12} and renders them non-ultrafiltrable through cellophane. The same or a similar substance appears also in the culture liquors, more especially in those from aged cultures.

Culture liquors of *Euglena gracilis* also have the property of sequestering vitamin B_{12}. This may be connected with Kristensen's (1955) finding that *Euglena* culture liquors contain a thermolabile factor which inhibits growth of the organism in a vitamin B_{12} limiting medium.

REFERENCES

Coates, M. E. & Ford, J. E. (1955). *Biochem. Soc. Symp.* In the Press.

Kristensen, H. P. Ø. (1955). *Acta physiol. Scand.* **33**, 232.

A Block in Arginine Synthesis in *Neurospora crassa* due to Gene Mutation. By J. R. S. Fincham and J. B. Boylen. (*Department of Genetics, University College of Leicester*)

Arginine-requiring mutant strains of *Neurospora crassa* were first studied by Srb & Horowitz (1944). These authors described one type of mutant which required arginine specifically, failing to respond to citrulline. A second such mutant type, genetically different from the first, has recently been discovered (D. Newmeyer, personal communication), and the present paper is concerned with the biochemical characteristics of this type.

Four strains, which so far appear to be identical, have been studied. All of them were found to accumulate argininosuccinic acid in the mycelium, and this accumulation was considerably enhanced by the addition of citrulline to the growth medium. Citrulline added to the mutant cultures could be almost quantitatively accounted for at the end of growth as residual citrulline plus argininosuccinic acid, while citrulline added to parallel wild-type

cultures completely disappeared. This accumulation of argininosuccinic acid by mutant mycelium in the presence of citrulline was accompanied by a marked depletion of the amino acids (predominantly alanine, glutamine and glutamic acid) normally present in mycelium.

Cell-free extracts of wild-type mycelium were found to contain an enzyme catalysing the reversible splitting of argininosuccinic acid to arginine and fumaric acid. This enzyme was purified fivefold by ammonium sulphate fractionation. The mutant strains appeared to lack this enzyme; no activity could be found either in crude extracts or in ammonium sulphate fractions, although as little as 0·2 % of the normal wild-type activity should have been detectable if present. Extracts of the mutant strains contained no detectable inhibitor of the enzyme. Arginase and fumarase were produced by the mutants in normal amounts. This case appears to be a straightforward one of a gene mutation having its effect through affecting the production of a single enzyme.

The results indicate that the mechanism of the conversion of citrulline to arginine in *N. crassa* is similar to that demonstrated by Ratner and her collaborators (Ratner, Petrack & Rochovansky, 1953; Ratner, Anslow & Petrack, 1953) for mammalian tissues.

REFERENCES

Ratner, S., Anslow, W. P. & Petrack, B. (1953). *J. biol. Chem.* **204**, 115.

Ratner, S., Petrack, B. & Rochovansky, O. (1953). *J. biol. Chem.* **204**, 95.

Srb, A. M. & Horowitz, N. H. (1944). *J. biol. Chem.* **154**, 129.

The Detection of 16-Oxooestradiol-17β in the Urine of Pregnant Women. By Elizabeth J. D. Watson and G. F. Marrian. (*Department of Biochemistry, University of Edinburgh*)

Following the isolation of 16-*epi*oestriol from the urine of pregnant women, Marrian & Bauld (1955) tentatively suggested that the common metabolic precursor of both *epi*oestriol and oestriol might be 16-oxooestradiol-17β (cf. Huffman & Grollman, 1947). A systematic search for 16-oxooestradiol-17β in the urine of pregnant women has now been conducted.

Ether-soluble 'neutral and phenolic' fractions from acid-hydrolysed pregnancy urine were treated with Girard's Reagent T by refluxing in ethanol-acetic acid solution. From the ketonic fractions thus obtained the phenolic-ketonic fractions were separated in the usual way. Such phenolic-ketonic fractions were partitioned on Celite columns with 70 % methanol as stationary phase and benzene-ethylene dichloride (75:25) as mobile phase.

These chromatograms revealed the presence in the urinary extracts of two different ketonic Kober-chromogens. The elution pattern of the less 'polar' of these was indistinguishable from that of oestrone in the same solvent system, while the elution pattern of the more 'polar' one was indistinguishable from that of synthetic 16-oxo-oestradiol-17β prepared by the method of Huffman & Lott (1948). In three experiments on different samples of late pregnancy urine the yields obtained of the 'more polar' Kober-chromogen were 81, 307 and 307 μg./24 hr. respectively.

The phenolic-ketonic fraction from 200 l. of late pregnancy urine was subjected to a 10-transfer countercurrent distribution in separating funnels using the solvent system 50 % ethanol-benzene: hexane (80:20). The observed distribution of Kober-chromogens closely resembled the theoretical distribution for a mixture of oestrone and 16-oxooestradiol-17β which was calculated from the determined partition ratios of these compounds (oestrone, K = 9·8; 16-oxooestradiol-17β, K = 0·95).

In view of this evidence it is believed that the 'more polar' ketonic Kober-chromogen is identical with 16-oxooestradiol-17β.

Yields of 16-oxooestradiol-17β from late pregnancy urine were increased 2- to 3-fold by substituting incubation at pH 4·6 with an enzyme preparation from *Patella vulgata* for the usual preliminary acid hydrolysis of the urine. Subsequently it was found that although 16-oxooestradiol-17β is stable to boiling for 60 min. with 15 vol. % of 10 N-HCl in aqueous solution, it undergoes extensive destruction when subjected to the same treatment after solution in urine. It was also found that 16-oxooestradiol-17β undergoes extensive destruction when heated in ethanolic solution with acetic acid and Girard's Reagent T. It is concluded, therefore, that the amounts of 16-oxooestradiol-17β determined in the early experiments must have represented only small fractions of those that were actually present in the urine.

REFERENCES

Huffman, M. N. & Grollman, A. (1947). *Endrocrinology*, **41**, 12.

Huffman, M. N. & Lott, M. H. (1948). *J. biol. Chem.* **172**, 325.

Marrian, G. F. & Bauld, W. S. (1955). *Biochem. J.* **59**, 136.

The Reaction of L-Histidine with D-Glucose. By S. LEWIN. (*Physical Chemistry Laboratory, South-West Essex Technical College, London, E.* 17)

The reaction of histidine with glucose has been noted (Ågren, 1940; Kubota, 1941), but no systematic investigation was carried out to determine its mechanism.

We have investigated, aerobically and anaerobically, this combination using varying concentrations (M/15 to M/62·5 L-histidine, and up to 1·2M D-glucose) over the pH range 5–9, and at various temperatures (20°, 30°, 40° and 50°) by following the resulting pH depressions using glass electrode measurements.

As soon as the glucose and histidine solutions were mixed, the pH of the mixture dropped, eventually reaching a steady value. This was reached within a week at 20° and at the higher temperatures in shorter periods, requiring less than 2 hr. at 50°. The results obtained in this steady pH state, over the approximate pH range of 8–9, have been found to be in accord with a mechanism involving one glucose molecule and one anionic histidine molecule, similar to that proposed by Katchalsky (1941) for several other amino acid-glucose combinations. No combination was detected over the pH range of 5–6·5. The value of the equilibrium constant decreased with rise in temperature from 3·9 at 20° to 2·3 (±0·2) at 50° respectively, thus showing the reaction to be exothermic.

The life-time of the above first steady pH state decreased with rise in temperature; it lasted about 4 weeks at 20°, but only about 5 hr. at 50°. It was followed by a further pH drop leading eventually to a second steady pH state. At the higher temperatures several consecutive steady pH states were found.

The development of brown colour, as associated with the 'browning reaction', was observed visually at the end of the second, or the beginning of the third, steady pH state, and its intensity increased with the passage of time. Less intensity of colour was found under aerobic than under anaerobic conditions. Increase in initial pH and glucose concentration resulted in the browning becoming more pronounced. No browning was noted even after 8 months when the glucose-histidine mixtures were kept below 0°.

REFERENCES

Ågren, G. (1940). *Acta physiol. Scand.* **1**, 105.
Katchalsky, A. (1941). *Biochem. J.* **35**, 1024.
Kubota, T. (1941). *J. Biochem.* (*Japan*), **34**, 119.

DEMONSTRATION

The Pitcher Secretion of *Nepenthes*. By SHELAGH MORRISSEY and MARGARET M. MURRAY. (*Departments of Botany and Physiology, Bedford College, University of London*)

A close parallelism has been shown between the secretion of the pitcher plant *Nepenthes* and that of the mammalian stomach.