PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **222nd Meeting** *of the Biochemical Society was held in the Department of Physiology, The University, Manchester, on Friday,* 10 *July* 1942, *at* 2.15 *p.m., when the following papers were read:*

The Anaphylactic Sensitization of Guinea-pigs to different Proteins. By L. B. WINTER

Some Aspects of Polysaccharide Storage in *E. coli*. By J. DAWSON and F. C. HAPPOLD

Evans, Handley & Happold [1941]* demonstrated that the glucose inhibition of the tryptophanase system in *E. coli* depended on the presence of phenylalanine or tyrosine in the cultural medium and suggested [1942]† that this inhibition depended on increased polysaccharide storage in the organism. *E. coli* has been grown on two types of glucose-tryptophane media, only one of which contained *dl*-phenylalanine. Determinations of the polysaccharide content of the organisms grown on both media have been carried out according to the method of Sahyun [1931].‡ The polysaccharide content of the cells grown in the phenylalanine-containing medium was greater than that of those grown in the phenylalanine-free medium. Additional confirmation of these results was obtained from respiration experiments. Similar polysaccharide determinations have been carried out on cells grown in media in which the sugar was either mannose or ribose. The polysaccharide content of these cells was lower than that of cells grown in a similar glucose medium, and also the addition of phenylalanine to the cultural medium did not increase the polysaccharide content of the cells.

The effect of *l*(−)-phenylalanine on polysaccharide storage has also been determined. This isomer was incapable of increasing the polysaccharide content of *E. coli* and also unable to bring about the glucose inhibition of the tryptophanase system.

* Evans, Handley & Happold [1941]. *Biochem. J.* **35**, 207.
† Evans, Handley & Happold [1942]. *Biochem. J.* In the Press.
‡ Sahyun [1931]. *J. biol. Chem.* **93**, 227.

The Nutrition of *C. diphtheriae* (intermediate type). By F. W. CHATTAWAY, F. C. HAPPOLD, B. LYTHGOE, M. SANDFORD and A. R. TODD

In continuation of the studies on the nutrition of *C. diphtheriae* growth factors for intermediate types were sought and found in the amyl alcohol-insoluble fraction from Glaxo first and second liver filtrate residues. This activity was not removed by adsorption on to fuller's earth at *p*H 2·0. Adsorption results with norite were variable. Extraction with butanol gave activity in both soluble and insoluble fractions, the greater being in the latter. There was no loss of activity when the active preparation was heated with 10 % H_2SO_4 for 2 hr. at 100° C., but similar treatment with 5 % NaOH resulted in complete loss in activity. Activity was also largely destroyed by methylation or acetylation or by treatment with nitrous acid. Phosphotungstic acid precipitation left the bulk of the active component in the filtrate.

The Nutrition of *C. diphtheriae gravis* (Dundee). By F. W. CHATTAWAY, F. C. HAPPOLD, B. LYTHGOE, M. SANDFORD and A. R. TODD

A basal medium which contains pimelic acid, nicotinic acid, β-alanine and pantothenic acid fails to support the growth of *gravis* (subtype Dundee). Growth is restored by the addition of the amyl alcohol-insoluble fraction from Glaxo first or second liver filtrate residues. The activity is partially destroyed by heating with NaOH to *p*H 11 at 100° for 2 hr. It is precipitated by basic lead acetate, adsorbed on to norite at *p*H 3·0 and 9·0 but not near to neutrality. Activity is largely destroyed by methylation and acetylation. It is not extracted by butanol from concentrated aqueous solution and it is insoluble in 100% ethanol. It is not adsorbed on to fuller's earth from acid solution *p*H 3·0, nor is it destroyed by tryptic nor peptic digestion.

The original preparation was also shown to be biotin active for yeast (Gebrüder Meyer), but this growth-stimulating effect was suppressed by avidin, which had, however, no action upon the growth-stimulating effect for *C. diphtheriae gravis* (Dundee). Neither crystalline biotin methyl ester, nor biotin promotes growth of the Dundee strain in the basal medium, but this occurs in the presence of *p*-amino benzoic acid. Separation of partially purified liver fractions by chromatogram technique indicates the probable presence of *p*-amino benzoic acid.

The Nutrition of *Lactobacillus casei* E. By F. W. CHATTAWAY, F. C. HAPPOLD, B. LYTHGOE, M. SANDFORD and A. R. TODD

The original basal medium of Strong and Snell for the estimation of riboflavine in pantothenic acid using *Lactobacillus casei* E gave very uneven results.

Using an acid hydrolysate of casein, adsorbed with norite at acid *p*H, good growth and acid production can be obtained by addition of the amyl alcohol-insoluble fraction from Glaxo first residues. The active fraction is not extracted from the concentrate by butanol nor by fuller's earth at *p*H 2·1. This treatment removes all traces of riboflavine which must be returned to the medium. The addition of *p*-amino benzoic acid with low concentrations of the fuller's earth treated liver extract increases growth and acid production. *p*-Amino benzoic acid is almost certainly present in the active liver fraction. Adenine, guanine and uracil are without effect on the growth of *Lactobacillus casei* E, both in the presence and absence of biotin.

The *p*-Aminobenzoate of Plasma in Relation to the Action of Sulphanilamide. By H. MCILWAIN

For antistreptococcal activity *in vitro*, sulphanilamide is required in concentrations at least 5000 times those of the coincident *p*-aminobenzoate.* Some observations have suggested that this ratio might not hold *in vivo*.† As study of antagonistic agents appears a promising way of correlating *in vitro* and *in vivo* actions of drugs, the *p*-aminobenzoate of plasma and some other materials has been determined to see if it is consistent with this ratio (the antistreptococcal index‡ of the drug) and with the therapeutic activity of sulphanilamide.

Plasma specimens, assayed with *Clostridium acetobutylicum*, showed activity corresponding to $10^{-8}M$ and less *p*-aminobenzoate. The blood level of sulphanilamide during treatment is usually $1{\cdot}5\text{–}6 \times 10^{-4}M$, i.e. at least 3–12 times that necessary to antagonize such *p*-aminobenzoate concentrations. Plasma can, however, contain 100 times this

concentration of *p*-aminobenzoate, estimated as above, in a form available to *Cl. acetobutylicum* and *Streptococcus haemolyticus* after digestion with acid. *p*-Aminobenzoate could therefore be the sulphonamide antagonist of autolysed tissues and of bacteriological media in which sulphanilamide is inactive.

* Woods, D. D. [1940]. *Brit. J. exp. Path.* **21**, 74. Cf. also Wood, W. B. [1942], *J. exp. Med.* **75**, 369 and Harris, J. S. & Kohn, H. I. [1941], *J. Pharmacol.* **73**, 383.
† For example, Selbie, F. R. [1940]. *Brit. J. exp. Path.* **21**, 90.
‡ McIlwain, H. [1942]. *Brit. J. exp. Path.* **23**, 95.

Principles of Fluorimetric Estimation of Vitamins. By F. WOKES, J. G. ORGAN and F. C. JACOBY

Quantitative studies, using the Spekker fluorimeter, have been made of the fluorescence of pure thiochrome* in *iso*butanol, pure quinine acid sulphate† in $N/10$ H_2SO_4, and eosin and fluorescein in phosphate buffer at *p*H 8. For each of these there is an *optimal* concentration at which Beer's law is most nearly obeyed, and a *maximal* concentration at which the fluorescence reaches its maximum. Concentrations above and below the optimal show deviations from Beer's law which become greater as the concentration departs further from the optimal. The results confirm and extend the earlier observations of Kuhn‡ and indicate the desirability of using calibration curves and solutions of which the concentration is not too far from the optimal. Deviations from Beer's law may also be due to quenching by other fluorescent substances present in the solution. The degree of quenching is affected by the concentration of quenching substance, and if correction is not made serious errors may occur in assays giving high 'blanks'. Possible methods of correcting for this quenching are discussed.

* From Merck and Co. of New York. † From Dr T. A. Henry.
‡ Hauser, K. W., Kuhn, F. & Kuhn, E. [1935]. *Z. phys. Chem.* **29**, 417.

The 'Catalase Test' with Special Reference to *Acetobacter* Species. By T. K. WALKER and J. TOŠIĆ

Ten authentic cultures of different *Acetobacter* species and nine newly isolated strains of organisms of the same genus proved to be catalase-positive if examined in the early logarithmic phase of growth before acid formation had become pronounced. Older cultures of the same organisms gave a doubtful catalase reaction and some even a negative reaction. When the malt-extract agar medium was suitably buffered by calcium carbonate these irregularities were no longer observed and all the species examined gave a positive catalase reaction, even 14 days after inoculation. This sensitivity to acid has a parallel in the observation of K. R. Butlin [1938]* that *A. suboxydans* contains as one of its enzyme components an acid-sensitive highly oxidizing system which, in an unbuffered medium, is readily inactivated if the substrate yields an acid product (e.g. gluconic acid).

Estimations have been made of the relative amounts of catalase produced by eleven species of *Acetobacter* in pure culture.

* Butlin, K. R. [1938]. *Biochem. J.* **32**, 508, 1187.

***Acetobacter* Species as Common Contaminants of Brewery Yeast.** By T. K. WALKER and J. TOŠIĆ

Many samples of brewing yeasts collected from different sources proved on examination to be strongly infected by *Acetobacter* species, and in the majority of cases more than one species was found in association with one sample of yeast. By suitable processes a

number of *Acetobacter* species was isolated in pure culture and some of these were found to be species already described. In addition, three hitherto unknown species, possessing very distinctive features, were isolated and have been designated *A. turbidans*, *A. acidum-polymyxa* and *A. anulogenes* respectively. The first-mentioned was described recently by A. J. C. Cosbie and the present authors [1941, 1942].* Descriptions of the two other new organisms will be recorded in future communications.

So far as we are aware it has not been realized previously that many *Acetobacter* species live in association with brewery yeast, and it would appear that such yeast may prove a fruitful source from which to obtain new and interesting members of the genus *Acetobacter*.

These observations render it evident that when brewing yeast is used as a reagent for the purpose of effecting a chemical change of biological significance, it is important to use yeast grown in pure culture, since a microscopical examination of brewing yeast is not sufficient always to reveal the presence of *Acetobacter*, and special methods are necessary to detect these organisms.

* Cosbie, A. J. C., Tošić, J. & Walker, T. K. [1941]. *J. Inst. Brew.* **47**, 382; [1942], *J. Inst. Brew.* **48**, 82.

The Ornithine Cycle of Urea Formation. By H. A. Krebs

Leuthardt [1938]* found that liver of starved guinea-pigs forms urea more rapidly from glutamine than from NH_4Cl and concluded that NH_3 cannot be an intermediate in the urea formation from glutamine. It is shown that the rate of urea formation from NH_4-glutamate, or NH_4-lactate is the same as that from glutamine. The data are in agreement with the assumption that glutamine is hydrolysed to NH_4-glutamate and that this is followed by the ornithine cycle. The rapid urea formation from glutamine is due to a stimulating effect of glutamate on the ornithine cycle. The effect is probably similar to that of lactate and other oxidizable substances described by Krebs & Henseleit [1932].†

Bach [1939]‡ proposed two new mechanisms of urea synthesis in liver: the oxidation of citrulline to glutamic acid and urea, and the oxidation of ornithine to glutamine 'where the pathway joins that suggested by Leuthardt'. Bach's experimental results are not confirmed. His schemes are considered superfluous as the known facts are accounted for by the theory of the ornithine cycle.

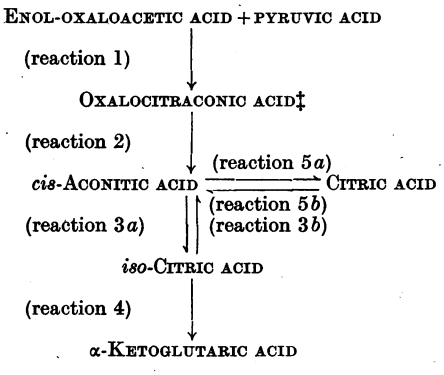
Trowell [1942],§ working on the perfused rat liver, observed that the rate of urea formation from arginine was sometimes lower than the rate of urea formation from NH_4Cl plus ornithine and that the effect of citrulline on urea formation was transitory and not catalytic. He concluded that the theory of the ornithine cycle is untenable. It is pointed out that the concentration of arginine in Trowell's experiments was only about 1/10 of that of NH_4Cl. Under these conditions the slow rate of urea formation from arginine is irrelevant. The 'transitory' nature of the citrulline effect was probably due to the exhaustion of the added NH_4Cl. Under suitable conditions citrulline has a 'permanent' and catalytic effect. The theory does not postulate that catalytic effects are observed under all conditions. The absence of catalytic effects can be due to side-reactions of ornithine, citrulline and arginine in which these amino acids are used up, e.g. the synthesis of proteins, the oxidative degradation and the conversion into other amino acids.

The ornithine cycle is in agreement with all experimental observations concerning the synthesis of urea from ammonia in liver. So far there is no reason to postulate alternative mechanisms of urea synthesis in mammalian liver.

* Leuthardt, F. [1938]. *Hoppe-Seyl. Z.* **252**, 238.
† Krebs, H. A. & Henseleit, K. [1932]. *Hoppe-Seyl. Z.* **210**, 33.
‡ Bach, S. J. [1939]. *Biochem. J.* **33**, 1833.
§ Trowell, O. A. [1942]. *J. Physiol.* **100**, 432.

Modified Citric Acid Cycle. By H. A. KREBS

Wood, Werkman, Hemingway & Nier [1941]* and Evans, Jr. & Slotin [1941],† using carbon isotopes as tracers, have shown that citrate cannot be an intermediate when α-ketoglutarate is synthesized from pyruvate in pigeon liver. Wood *et al.* point out that the following modification of the citric acid cycle hypothesis is in accordance with the experimental observations:



According to the earlier scheme oxaloacetate and pyruvate react to form first citrate and then *cis*-aconitate. The modified hypothesis suggests that *cis*-aconitate is formed more directly and that the formation of citrate is due to a side reaction. Evans, Jr. & Slotin consider the modified scheme as 'improbable in view of the demonstrated equilibrium between citrate, *iso*-citrate and *cis*-aconitate in most tissues'. Evidently this argument only holds if the equilibrium is actually established. Whether this is the case depends on the rates of the reactions (2), (3*a*), (3*b*), (4), (5*a*) and (5*b*) in the metabolizing tissue. Measurements of the rates of (3*a*) and (5*a*) show that at low concentrations of *cis*-aconitate (0·001 *M*) (3*a*) is about 10 times more rapid than (5*a*). With increasing concentrations of *cis*-aconitate the difference in the rates becomes smaller; at 0·003 *M* (3*a*) is 4 times, at 0·2 *M* 1·5 times more rapid than (5*a*). It is also shown that (4) is rapid as compared with (3*b*). The data indicate that the equilibrium between citrate, *iso*-citrate and *cis*-aconitate is not established in the metabolizing tissue owing to the relative slowness of the reactions (5*a*) and (5*b*). The objections of Evans, Jr. & Slotin against the modified hypothesis are therefore not valid.

* Wood, H. G., Werkman, C. H., Hemingway, A. & Nier, A. O. [1941]. *J. biol. Chem.* **139**, 483; [1942] *J. biol. Chem.* **142**, 31.

† Evans, E. A. Jr. & Slotin, L. [1941]. *J. biol. Chem.* **141**, 439.

‡ Wood *et al.* wrote 'pyruvyl-fumaric acid', i.e. the corresponding trans-isomeride. If the resulting aconitic acid possesses the *cis*-configuration it is almost certain that the immediate precursors have the same configuration. The 'enol oxaloacetic acid' is therefore expected to be hydroxy-maleic acid.

§ Krebs, H. A. & Johnson, W. A. [1937]. *Enzymologia*, **4**, 148.

The Formation of Viscous Materials by *Clostridium butylicum*. By B. LYTHGOE and J. MADINAVEITIA

McClean* has shown that the presence of hyaluronidase in the culture media of certain strains of capsulated streptococci prevents the formation of capsules, and that addition of hyaluronidase to normal cultures causes the disappearance of the capsules. The polysaccharide constituent of these capsules is probably identical with hyaluronic acid.

In connexion with other work we observed that *Cl. butylicum* produces a viscous material when grown in a relatively simple medium (glucose, asparagin and inorganic

salts supplemented by bactopeptone as a source of biotin).† Although the viscous material produced by *Cl. butylicum* is not hyaluronic acid, its formation by the organism as well as the growth of the latter are inhibited to a certain extent by the presence of hyaluronidase concentrates in the culture medium. The formation of viscous material by this micro-organism is much more markedly affected by the concentration of glucose and bactopeptone in the medium than by the presence of hyaluronidase.

The optimal concentrations of glucose and of peptone for bacterial growth do not coincide with those optimal for production of viscous material. There is an optimal concentration of peptone for the formation of viscous material; at higher concentrations the viscosity of the culture medium is not appreciably altered, although the optimal concentration of peptone for bacterial growth had not been reached in our experiments.

* McClean, D. [1941]. *J. Path. Bact.* **53**, 13.

† McDaniell, L. E., Woolley, D. W. & Peterson, W. H. [1939]. *J. Bact.* **37**, 259.