PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **Annual General Meeting** *of the Biochemical Society was held in the Department of Biochemistry, University College, Gower Street, London, W.C.* 1, *on Friday,* 24 *March,* 1950, *when the following papers were read:*

COMMUNICATIONS

Separation of *N*-2:4-Dinitrophenylamino-Acids by Partition Chromatography on Paper.

By S. Blackburn and A. G. Lowther. (*Wool Industries Research Association, Torridon, Headingley, Leeds*)

N-2:4-Dinitrophenylamino-acids (DNP amino-acids) are of importance in determining the 'end-groups' of peptides and proteins, and can be separated on partition chromatograms on silica gel using water or other solvent as the stationary phase (Sanger, 1945). Effective separations of DNP amino-acids can also be effected on silica gel columns using phosphate buffers instead of water as the stationary phase, with solvents such as ether as the mobile phase (Blackburn, 1949).

In contrast to some large DNP peptides which appear to move satisfactorily on paper chromatograms, DNP amino-acids have generally given unsatisfactory results, due to the marked 'tailing' of the spots which is observed. Phillips & Stephen (1948) reported some success using a two-dimensional method, particularly with the slower moving DNP amino-acids, but also observed marked 'tailing' in many solvents.

We have now observed that DNP amino-acids may be successfully separated on one-dimensional paper partition chromatograms using paper previously soaked in phthalate or citrate buffers and dried in air, solvents used being saturated with buffer at room temperature. The DNP amino-acids then move down the paper as compact 'spots' with characteristic rates (R_F values), 'tailing' being eliminated.

A number of solvents have proved suitable. 1% butanol-chloroform is a suitable solvent for the separation of the faster moving DNP monoamino monocarboxylic acids, while ethyl acetate or *n*-butanol separate the slower moving DNP amino-acids from each other. The method has a high resolving power, a mixture of DNP-leucine, DNP-valine, DNP-alanine and DNP-glycine separating into four clearly defined spots in 30% propanol-cyclohexane. The possibility of performing end-group estimations on a micro scale is thus opened up.

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The Effect of *n*-Alkyl 3:5-Diiodo-4-hydroxybenzoates on Oxygen Consumption in Mice.

By J. H. Wilkinson, M. M. Sheahan and N. F. Maclagan. (*Department of Chemical Pathology, Westminster Medical School, London, S.W.* 1)

The work of Frieden & Winzler (1949) on the effect of thyroxine analogues on metamorphosis in tadpoles has directed attention to the inhibitory properties of simpler compounds such as 3:5-diiodo-4-benzyloxybenzoic acid. Compounds of this type have also given results on oxygen consumption in mice suggesting active competition with thyroxine (Maclagan, Sheahan & Wilkinson, 1949). We have extended this work by the preparation of a series of new *n*-alkyl esters of 3:5-diiodo-4-hydroxybenzoic acid, which were tested for their effect on oxygen consumption in thyroxinized mice by a method described elsewhere (Maclagan & Sheahan, 1950). Inhibitory activity in this series has been demonstrated up to the *n*-octyl ester, the lower homologues, particularly the ethyl, propyl and butyl esters, being the most active. For example, the butyl ester produced a 62% inhibition of the thyroxine effect in a total dosage of 50 mg./kg. body weight (thyroxine dosage 2 mg./kg.).

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Utilization of Acetate for Milk-fat Synthesis in the Lactating Goat. By G. POPJÁK, T. H. FRENCH and S. J. FOLLEY. (*The National Institute for Medical Research, The Ridgeway, London, N.W. 7, and The National Institute for Research in Dairying, University of Reading*)

Synthesis of fatty acids and cholesterol has been shown to occur in the mammae of non-lactating pregnant rabbits (Popják & Beeckmans, 1950). It was also found that the short-chain volatile fatty acids obtained from the mammae of such rabbits are derived by synthesis from acetate and not by degradation of the long-chain fatty acids (Popják, Folley & French, 1949). The *in vivo* utilization of acetate for milk fat synthesis (particularly for the synthesis of volatile acids) confirmed the suggestion of Folley & French (1949*a*, *b*) based on their *in vitro* studies of lactating mammary tissues.

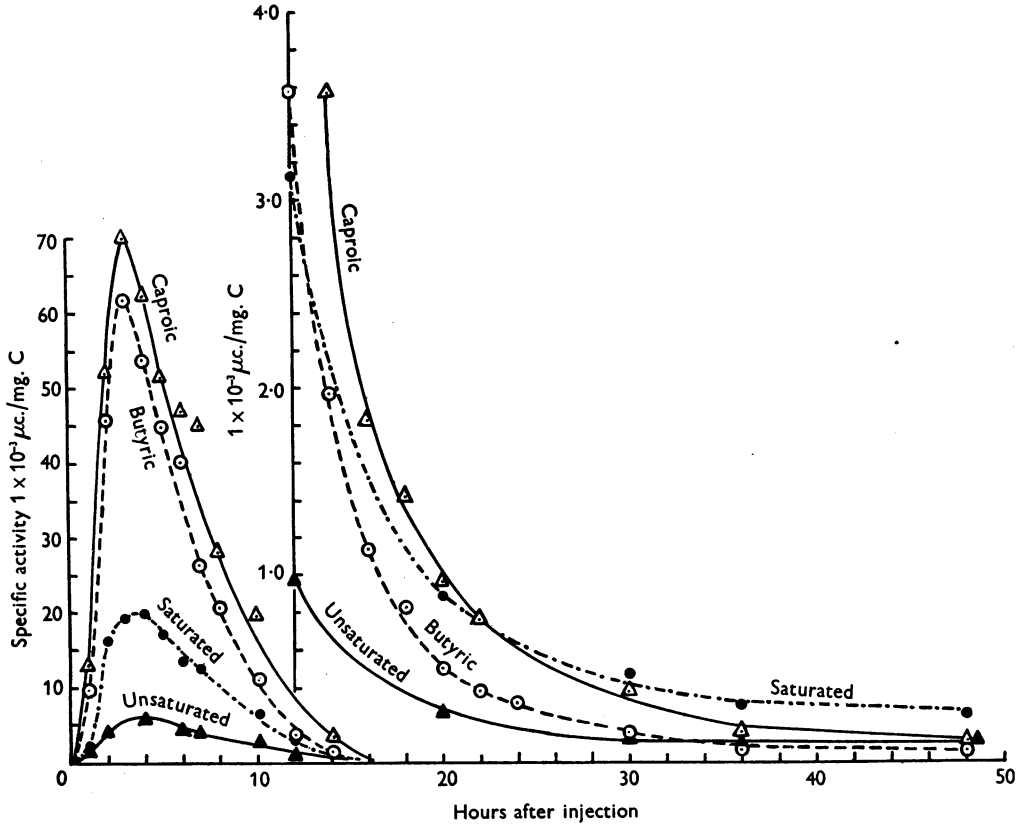

Fig. 1.

In the present experiment milk-fat formation was investigated with the aid of $CH_3{}^{14}CO_2Na$ in a lactating goat. The animal was injected intravenously with a single dose of 5 mc. of ^{14}C contained in 430 mg. of $CH_3{}^{14}CO_2Na$ dissolved in 0·9 % NaCl. Respiratory CO_2 was collected continuously for 6 hr. after the injection and the animal was milked at frequent intervals for 48 hr., after which time it was killed. The glyceride fatty acids extracted from the milk were separated into steam-volatile and non-volatile acids, the former having been further divided into water-soluble and insoluble fractions. The non-volatile acids were fractionated into saturated and unsaturated fatty acids by the lead salt method (Twitchell, 1921). The ^{14}C content of the fatty acids was then determined and is shown in Fig. 1. The specific activity-time curves of the milk fatty acids reached their maxima 3 hr. (volatile acids) and 4 hr. (non-volatile acids) after the injection and declined exponentially thereafter with a half-life of about 4 hr. Since the specific activity of the volatile fatty acids at their maxima was several times that of the long-chain non-volatile acids, it is quite clear that the volatile acids could not have originated from the degradation of the long-chain fatty acids but must have been synthesized from acetate or a derivative

of it. The maximum specific activity of the plasma fatty acids was reached at about 24 hr. after the injection of the labelled acetate. At this time the milk fatty acids still contained more ^{14}C than the plasma fatty acids. If one were to determine the specific activity of plasma fatty acids 3–4 hr. after the injection by extrapolation from the specific activity-time curve, it would be found that the specific activity of all fatty acid fractions in the milk at this time was several hundred times that of the plasma fatty acids. Hence, it must be concluded that synthesis from small molecules in the udder is of outstanding importance in milk-fat formation. Further work is being planned to obtain individual fatty acids from our material and to carry out chemical degradation of these in order to obtain evidence as to the mechanism of fatty acid synthesis.

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Some Observations on the Adreno-cortical Steroids in Human Urine. By J. Y. F. Paterson, R. I. Cox and G. F. Marrian. (*Department of Biochemistry, University of Edinburgh*)

There are two general types of method for the estimation of urinary adrenal cortical steroids. One type of method estimates these steroids by the reducing power of suitably prepared extracts of urine (Talbot, Saltzman, Wixom & Wolfe, 1945; Heard & Sobel, 1946; Heard, Sobel & Venning, 1946). The other type of method estimates these steroids by the formaldehyde generated on oxidation of urine extracts with periodic acid (Lowenstein, Corcoran & Page, 1946; Daughaday, Jaffe & Williams, 1948). By either type of method the amount of adrenocortical steroids which can be extracted from urine is increased by preliminary acidification of the urine. This suggests that these steroids may be excreted, at least in part, as conjugates.

In the present work the conditions necessary for the optimum hydrolysis of these presumed conjugates were studied, using a slightly simplified Daughaday method. Urine was acidified to pH 1, allowed to stand at 25°, and duplicate aliquots withdrawn for estimation at various time intervals. Estimations were also done on aliquots of the urine which had not been acidified. The hydrolysis curve so obtained was unexpected (Fig. 1, solid points), and indicates that the formaldehydogenic substances estimated by this method may be divided into two groups. The first group is acid labile, and the other stable to acid under the conditions used.

Heard *et al.* (1946) have observed that when urine is acidified, the amount of 'reducing substance' which can be extracted by chloroform-ether is increased. If, before extraction, the urine is neutralized immediately after acidification, the amount of reducing substance extracted is nearly the same as that extracted from unacidified urine. In the present work, hydrolysis curves, in which one of the duplicate aliquots was neutralized before extraction, were done. The results (see figure) confirm Heard's observations and indicate that both groups of formaldehydogenic substances are conjugated. The conjugates of the first (labile) group can be extracted by chloroform from acid urine, but not from neutral urine. The conjugates of the second (stable) group cannot be extracted by chloroform either from neutral or acid urine.

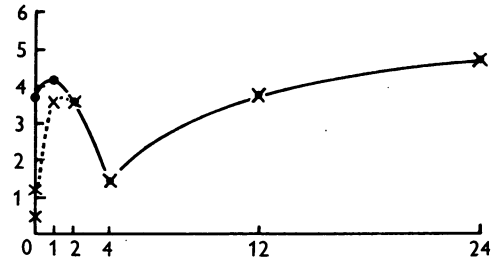

Fig. 1. Hydrolysis curve: normal male. Abscissa: time interval between acidification and extraction (solid points) or acidification and neutralization (followed immediately by extraction) (crosses) in hours. Ordinate: formaldehyde (μg.) per aliquot (1/15 of 24-hr. urine).

Increases in the amounts of both groups of substances have been found in pregnancy, in a case of Cushing's syndrome, and on administration of ACTH to a rheumatoid arthritic woman. Two abnormal hydrolysis curves have been observed.

The importance of the above results in routine estimations is stressed. Talbot uses extracts of unacidified urine; Heard *et al.*, Lowenstein *et al.* and Daughaday *et al.* extract immediately after acidification, while Mason & Sprague (1948) allow the urine to hydrolyse for 1–3 days. All of these are obviously not 'optimum hydrolysis conditions'.

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The Conversion of Carotene to Vitamin A in the Intestine of the Chick. By S. Y. Thompson, M. E. Coates and S. K. Kon. (*National Institute for Research in Dairying, University of Reading*)

It is now established that carotene is converted to vitamin A in the small intestine of the rat and of the pig (for references see Thompson, Ganguly & Kon, 1949). We have found that the same is true of the chick.

Day-old chicks received a diet of the following percentage composition, supplemented with 1000 B.S.I. units of vitamin D_3/kg.: weatings 25·5, ground wheat 20, ground oats 10, ground barley 10, casein 10, dried skim milk 6, groundnut meal 10, brewer's yeast 3, groundnut oil 2, $CaCO_3$ 3, NaCl 0·5. On this diet their vitamin A reserves were, as shown below, rapidly depleted and interference with growth became evident during the third week:

Age of chicks in days and number (in parentheses)	1 (2)	8 (2)	15 (1)	36 (4)	42 (1)
Vitamin A (i.u./liver)	11	22	2	1	2

The increase of liver reserves at 8 days was probably due to absorption of the vitamin from the yolk sac.

At 6 weeks some of the birds were starved overnight and then offered solid food for a few minutes before dosing by tube with 4 mg. β-carotene dissolved in 400 mg. groundnut oil. They were killed by decapitation 0·5, 1, 2 and 4 hr. after dosing. The small intestine was washed out immediately with saline. There was very little vitamin A in the contents. Fig. 1 shows that vitamin A, measured as described by Thompson *et al.* (1949), appeared in the intestinal wall within 0·5 hr., the shortest time interval studied, whereas the liver stores did not increase appreciably till 3·5 hr. later. For this reason chicks are likely to prove specially suitable for the study of the intestinal conversion of carotene. They are so easily rendered deficient that they may also be of value for the biological assay of vitamin A.

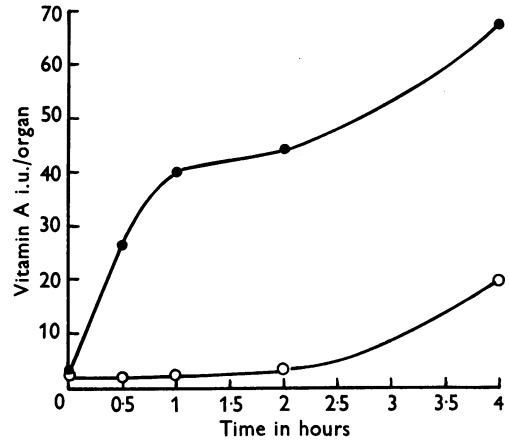

Fig. 1. Appearance of vitamin A in the small intestine (●—●) and liver (○—○) of vitamin A-deficient chicks after a meal of β-carotene. Mean values for two chicks.

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Conversion of Carotene to Vitamin A in the Intestine of the Pig and of the Rat: Transport of Vitamin A by the Lymph. By M. E. Coates, S. Y. Thompson and S. K. Kon. (*National Institute for Research in Dairying, University of Reading*)

In continuation of the work presented a year ago before this Society (Thompson, Braude, Cowie, Ganguly & Kon, 1949), plastic cannulas were placed in the mesenteric lymphatics of pigs and rats. For rats the technique was that of Bollman, Cain & Grindlay (1948), except that polythene, instead of polyvinyl chloride, tubing was used, and that the tube was pushed directly through the wall of the lymph vessel and not through a prepared hole. For pigs the procedure was essentially similar. After cannulation the rats were kept in the cage described by Bollman & Van Hooke (1948). Normal rats

were anaesthetized with Nembutal (Abbott Laboratories); those made vitamin A-deficient on the diet of Thompson, Ganguly & Kon (1949) were given ether.

In pigs, whether β-carotene (300 mg.) or vitamin A (100,000 i.u.) was given, the marked (100-fold) increase in the concentration of vitamin A in the lymph was almost exclusively in the ester form (cf. for carotene Thompson, Braude, Cowie, Ganguly & Kon, 1949; Thompson, Ganguly & Kon, 1949; for vitamin A, Drummond, Bell & Palmer, 1935; Eden & Sellers, 1948; Goodwin & Gregory, 1948).

In rats, whether normal or vitamin A-deficient, the increase was also mostly as ester. Vitamin A did not appear in the lymph in increased quantities for at least 1 hr. after the carotene meal; though conditions were not strictly comparable it is probably significant that we found it in the intestine within 15 min. of dosing (Thompson, Ganguly & Kon, 1949). In the vitamin A-deficient rat killed 2 hr. after the carotene meal appreciable quantities of vitamin A were present in the intestine and in the lymph collected between 1 and 2 hr. after the meal, but none appeared in the blood or in the liver. Yet in the uncannulated animal killed at the same time the increase in the blood and liver was marked (see Table).

Appearance of vitamin A in the small intestine, mesenteric lymph, blood and liver of vitamin A-deficient rats after a meal of about 4 mg. β-carotene in arachis oil. Animals dosed 3 hr. after cannulation of lymphatic

Treatment	Time after dosing (min.)	Small intestine total vitamin A (i.u./rat)	Lymph: Fluorescence in ultra-violet	Lymph: Yield (ml.)	Lymph: Vitamin A (i.u.) Alcohol	Lymph: Vitamin A (i.u.) Ester	Blood (i.u./rat) Alcohol	Blood (i.u./rat) Ester	Liver total vitamin A (i.u./rat)
Experiment 1									
Cannulated and dosed	0	—	Bluish	0·9	0·3	0·3	—	—	—
	30	—	Bluish	0·5	0·3	0·2	—	—	—
	60	—	Bluish	0·3	0·2	0·3	—	—	—
	75	12	Yellow	0·5	0·3	1·5	1·8	0·8	1·4
Not cannulated, arachis oil only	75	1·8	—	—	—	—	1·8	1·4	0·9
Experiment 2									
Cannulated and dosed	0	—	Bluish	2·0	0·2	0·2	—	—	—
	30	—	Bluish	0·5	0·2	0·1	—	—	—
	60	—	Bluish	0·7	0·2	0·1	—	—	—
	90	—	Yellow	0·7	0·4	4·0	—	—	—
	120	13	Yellow	0·8	0·4	8·6	1·4	0·9	2·1
Not cannulated, dosed	120	35	—	—	—	—	13·7	7·5	24·0
Not cannulated, arachis oil only	120	0·9	—	—	—	—	1·1	0·2	0·4

All these experiments, together with those reported earlier (Thompson, Braude, Cowie, Ganguly & Kon, 1949; Thompson, Ganguly & Kon, 1949), make it clear that the vitamin A arising from carotene in the intestine is carried thence by the lymph to the blood and by the blood to the liver.

We are greatly indebted to our colleagues: to Dr A. T. Cowie for the cannulation of the pigs, to Mr H. S. Hall for the special cages, to Dr K. M. Henry for the preparation of the rats and to Dr R. Braude for the preparation of the pigs.

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Ayfivin and Bacitracin. By G. G. F. NEWTON and E. P. ABRAHAM. (*Sir William Dunn School of Pathology, University of Oxford*)

The antibiotic ayfivin, produced by a strain of *Bacillus licheniformis*, was partially purified by Sharp, Arriagada, Newton & Abraham (1949). The product was found to be a mixture of polypeptides and to resemble bacitracin. Arriagada, Florey, Jennings & Wallmark (1949) showed that crude ayfivin cured mice of certain systemic infections, but that it severely damaged the kidneys. Further experiments are now reported on the separation of the components of the crude product and on the relationship of ayfivin to bacitracin (Gregory, Barry & Craig, 1948, 1949).

Method. The method of counter-current distribution between solvents, developed by Craig and co-workers, was used in the present investigations. Most of the distributions were carried out in a solvent-system composed of amyl alcohol, *n*-butanol and phosphate buffer, pH 7 (system I), but one comparative experiment was made using a mixture of *sec.*-butanol and aqueous acetic acid (system II). The material in different fractions was estimated by hydrolysis and measurement of the colour density given by the resulting amino acids when treated with ninhydrin in the manner described by Moore & Stein (1948). Initial distributions of several grams of material were carried out in separating funnels. Selected fractions were then subjected to 100 transfers in an apparatus of the type described by Craig & Post (1949).

Results. By distribution in system I crude ayfivin was resolved, or partly resolved, into at least seven polypeptides, of which three (referred to as A, B and C) had antibacterial activity. Substance A was the major active constituent. Its specific activity against *Corynebacterium xerosis* was similar to that of C and about four times that of B. It appeared to be stable on redistribution in system I.

In contrast to its behaviour in system I, crude ayfivin showed very little resolution on counter-current distribution in system II.

The distribution curve of commercial bacitracin in system I resembled that of partially purified ayfivin, and a mixture of ayfivin A and the corresponding component of bacitracin showed no sign of resolution into two substances after 100 transfers. The main active constituents of ayfivin and bacitracin are certainly very similar, and possibly identical. It is proposed to abandon the name ayfivin and to call its active constituents bacitracin A, B and C.

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Some Observations of the Fate of *bis(dimethylamino)-phosphonous anhydride* in the Rabbit. By J. E. GARDINER and B. A. KILBY. (*Department of Biochemistry, School of Medicine, University of Leeds*)

A dose of 50 mg./kg. of *bis(dimethylamino)phosphonous anhydride*, $(Me_2N)_2PO.O.PO(NMe_2)_2$, injected into rabbits leads to death within a few hours with typical symptoms of acetylcholine poisoning (excessive salivation, fibrillary twitchings, etc.) as produced by fluorophosphonates and other anti-cholinesterases. However, when the action of this anhydride on cholinesterases is measured *in vitro*, surprisingly high concentrations are required to produce 50% inhibition, in contrast with other organic phosphorus compounds which are effective at concentrations of the order of 10^{-7} to 10^{-8} M:

Source of cholinesterase	Conc. of anhydride for 50% inhibition (M)
Human erythrocytes	$1{\cdot}1 \times 10^{-1}$
Whole rabbit blood	$2{\cdot}6 \times 10^{-2}$
Bee brain (Metcalf & March, 1949)	$> 1{\cdot}2 \times 10^{-3}$

We have investigated this apparent anomaly by a comparison of the effects of the anhydride on rabbit blood cholinesterase activity *in vivo* and *in vitro*. A sample of anhydride incorporating ^{32}P has been made (Gardiner & Kilby, 1949) and injected into rabbits (50 mg./kg. i.p.) and the blood cholinesterase

activity at death measured manometrically. In parallel, blood withdrawn before injection was incubated at 37° with known concentrations of anhydride. The apparent concentration of anhydride in the blood *in vivo* was followed by radioassay of 0·1 ml. samples taken at 5–10 min. intervals:

	Rabbit I	Rabbit II
Inhibition of cholinesterase at death (%)	67·7	95·4
Apparent concentration of anhydride in blood at death from ^{32}P assay (mg./ml.)	0·044	0·15
Concentration of anhydride required to produce same inhibition *in vitro* (mg./ml.)	17·5	166

The inhibitory action of the anhydride on rabbit-blood cholinesterase is enhanced by previous incubation with rabbit liver slices:

Inhibitor solution added to blood	Inhibition of esterase. Final conc. of anhydride $5·5 \times 10^{-4}$ M	$2·8 \times 10^{-4}$ M
Supernatant from liver slices incubated in buffer solution (3 hr. 35°) + buffer solution containing anhydride (%)	6·7	7·6
Supernatant from liver slices incubated in buffer containing anhydride + buffer solution (%)	36·5	29·5

These results can be explained by postulating the conversion of the anhydride *in vivo* into some more active inhibitory compound, the liver being one place where this can occur. The formation of the half molecule, $(NMe_2)_2PO(OH)$, is excluded as this is inactive.

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Nitrogenous Excretion of Amphipods and Isopods. By Elisabeth I. B. Dresel and Vivien Moyle (introduced by E. Baldwin). (*Biochemical Department, University of Cambridge, and Marine Biological Laboratory, Plymouth*)

There exists among vertebrates a marked correlation between the predominant end-product of nitrogen metabolism and the availability of water. Amphibious and terrestrial species are ureo- or uricotelic, whereas species enjoying an abundant water supply are ammonotelic. Similarly, among invertebrates, the insects, which are essentially terrestrial, are uricotelic.

A group of amphipod and isopod Crustacea taken from wholly marine, marine littoral, estuarine, fresh-water and terrestrial habitats has now been studied. In all the species examined the predominant nitrogenous end-product was ammonia, which accounted for 50–90 % of the total non-protein nitrogen excreted. Urea and uric acid never accounted for more than 10 %.

That some form of metabolic adaptation has occurred in those species which are morphologically and physiologically adapted to terrestrial conditions is, however, apparent, for the total non-protein nitrogen output of the terrestrial species is only about one-tenth of that of the aquatic forms. Terrestrial adaptation in this group thus appears to be associated with an overall reduction of protein metabolism, a condition analogous to that which obtains in eggs of the cleidoic type.

Small amounts (5–10 %) of uric acid were found in the excreta of the terrestrial isopods and also of the fresh-water isopod, *Asellus aquaticus*. This uric acid might originate from the purines as a result of the loss of one or more uricolytic enzymes. It was accompanied by some retention of this insoluble compound, the amount so retained being correlated with the degree of adaptation to terrestrial conditions. An even greater retention, however, occurred in *A. aquaticus*.

Iodometric and Turbidimetric Determination of Mercapturic Acids in Urine. Mercapturic acid Excretion of Rabbits receiving Benzene. By D. V. PARKE and R. T. WILLIAMS. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2*)

Stekol (1936) estimated *p*-bromophenylmercapturic acid in rat and dog urine by hydrolysing the acid with 0·35N-NaOH to *p*-bromothiophenol which was determined iodometrically. In some cases the thiophenol was estimated gravimetrically as the mercuric mercaptide, but this method is inapplicable on a small scale. These methods, which are based on the original observations of Baumann (1882), are being investigated in detail.

Our studies have shown that mercapturic acids vary in their stability to alkali and are more stable than has been believed (see Table 1). Phenylcysteine is much more stable towards alkali than its *N*-acetyl derivative, phenylmercapturic acid, which is more stable than the halogen derivatives. In our estimations of mercapturic acids we used N-NaOH.

Table 1

Compound	Normality of boiling NaOH for complete hydrolysis in 30 min.
L-*p*-Chlorophenylmercapturic acid	0·4
L-*p*-Bromophenylmercapturic acid	0·4
L-Phenylmercapturic acid	0·75
L-*S*-Phenylcysteine	5·0

When mercapturic acids in urine are determined iodometrically, blank titrations are variable (cf. Binkley, 1949). In rabbits the blank variations can be reduced by careful selection of diets. However, in the phenylmercuric mercaptide method there is no blank, and it is more specific than the iodometric method. A turbidimetric procedure has been developed whereby mercapturic acid of the order of 1 mg. in 5 ml. urine can be estimated. In this method the opacity of suspensions of phenylmercuric mercaptide, produced in suitably treated urines by adding mercuric chloride, was estimated in the Spekker absorptiometer. Standard curves were made from suspensions of the mercuric mercaptide in 0·5N-Na_2SO_4 in 10% ethanol in water, stabilized with 0·01% gelatin. With urine gelatin is unnecessary.

With pure solutions of mercapturic acids, both methods give the same results, but in urine the iodometric method gives slightly higher results. Table 2 gives the mercapturic acid output in rabbits receiving benzene orally.

Table 2

Dose of benzene (g./kg.)	% dose excreted as mercapturic acid: Iodometric	Turbidimetric
1·0	1·6	1·3
1·0	0·8	0·65
1·0	1·2	0·8
1·0	0·75	0·6

Thus on the more specific turbidimetric method about 0·8% of the benzene appeared in the urine as a mercapturic acid (cf. Zbarsky & Young, 1943, for experiments on rats).

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A Colorimetric Assay Method for Vitamin B_{12}. By K. H. FANTES and D. M. IRELAND (*Research Division, Glaxo Laboratories, Ltd., Greenford, Middlesex*), and N. GREEN (*Research Division, Glaxo Laboratories Ltd., Barnard Castle, Co. Durham*)

A quantitative colorimetric method for assaying vitamin B_{12} in impure solutions could theoretically be based on (1) a change in the red colour of vitamin B_{12} to some different colour, or its specific decolorization, or (2) the colour of a hydrolytic product of vitamin B_{12} after preferential extraction to separate it from impurities.

Approach (1) was unsuccessful, although it was found that traces of chlorine in the presence of 5N-HCl would convert the red acid hydrolytic product of vitamin B_{12} to a substance with a blue-purple colour. The blue colour was formed by heating the vitamin B_{12} solution at 100° C. for 1 hr. with an equal volume of 10N-HCl, containing a trace of 16N-HNO_3 to generate chlorine. A slight excess of chlorine decolorized the solution, and the amount needed to produce the optimum blue colour was very critical. With impure preparations the amount of HNO_3 needed could not be predicted.

No oxidizing or reducing agents were found that specifically decolorized vitamin B_{12} or its acid hydrolytic product.

Lester Smith (1948) showed that the red hydrolytic product of vitamin B_{12}, an organic acid, was extracted by butanol. Ellis, Petrow & Snook (1949) mentioned the methyl ester of the acid. We have found that the red acid, on being shaken with a higher alcohol (e.g. octyl alcohol) in the presence of 5N-HCl, is slowly esterified, and that the ester so formed is quantitatively extracted into the alcohol. Addition of light petroleum does not transfer the ester to an aqueous or methanolic acid phase, but removes small amounts of coloured impurities.

The hydrolysis is done with an equal volume of 10N-HCl in sealed tubes at 100° for several hours. To convert the resultant organic acid to its ester it is necessary to shake the hydrolysate with an equal volume of *n*-octyl alcohol for 2 or 3 hr. An aliquot of the alcohol layer, diluted with up to seven volumes of light petroleum, is washed with a mixture of methanol:N-HCl (4:1). The colour of the octyl alcohol-petroleum solution is read in a 1 cm. or a 4 cm. micro-cell in a Hilger photoelectric absorptiometer with the use of an Ilford 604 filter.

Results were read from a standard curve. They were reproducible to ± 5%, and were in good agreement with those obtained by the microbiological method (Cuthbertson, 1949). Partly purified streptomyces fermentation liquors with a vitamin B_{12} content of 0·2% of the total solids and commercial liver extracts could be assayed by this method. The minimum amount of vitamin B_{12} that can be measured is about 40 μg. in not more than 2 ml. Related cobalt-containing factors of the vitamin B_{12} complex, such as vitamin $B_{12}b$ (Pierce, Page, Stokstad & Jukes, 1949), behave like vitamin B_{12} itself in this assay procedure.

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The Biogenesis of β-Carotene in the Fungus *Phycomyces blakesleeanus*. By G. A. Garton, T. W. Goodwin and W. Lijinsky. (*Department of Biochemistry, University of Liverpool*)

Phycomyces blakesleeanus is a very suitable organism with which to study carotenoid synthesis; it is easily cultured on liquid media and it produces only one carotenoid, β-carotene (Schopfer, 1935; Karrer & Krause-Voith, 1947). As little is known concerning carotenoid biogenesis, the first step was to determine quantitatively the production of carotene using the standard medium described by Schopfer (1934); this medium contains glucose (10%) as the C source and asparagine (0·2%) as the N source, together with KH_2PO_4 (0·15%), $MgSO_4.7H_2O$ (0·05%) and aneurin (25 μg. %). The cultures were incubated at 25° in a glass cabinet which received normal daylight illumination. The β-carotene was determined spectrophotometrically, and fat and dry-weight determinations were also made. The carotene content reached a maximum after 8–9 days and thereafter decreased quite rapidly; maximum dry weight and fat values were obtained after 3–4 days and thereafter remained constant. The results for the (+) and (−) strains of the fungus followed the same pattern, but the (+) strain produced only about half as much β-carotene as did the (−) strain, although the fat and dry weight production was of the same order. In further investigations only the (−) strain is being used.

When *Phycomyces* was cultured on the standard medium in the dark, β-carotene production was only about one-half of that produced in the light, whilst the fat and dry weight were not affected. These observations are contrary to those of Schopfer (1943) who reported no β-carotene formation in the absence of light.

When asparagine was replaced by L-valine, L-alanine or L-leucine no appreciable differences in carotene, fat or dry weight production could be detected, but replacement by glycine resulted in a marked and specific stimulation of carotenogenesis; L-*iso*leucine did not produce appreciable growth and NH_4NO_3 produced results similar to those obtained using asparagine until the pH of the medium fell below 3·4, when growth ceased.

Carotenogenesis appears to depend on the metabolism of exogenous nitrogen. If the culture medium is removed from a 3-day-old mycelium and replaced by a medium containing glucose but no nitrogen, fat and carbohydrate production proceeds normally, whereas β-carotene formation ceases.

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Chromatography of 2:4-Dinitrophenylhydrazones of Ketoacids on Alumina. By S. P. DATTA, H. HARRIS and K. R. REES. (*Department of Biochemistry and Galton Laboratory, University College, London*)

This method has been developed to investigate quantitatively the ketoacids in urine and other body fluids.

Alumina, 200 g. (Peter Spence Ltd., Type 'H'), is packed in ethyl acetate in a column 2·5 cm. in diameter. The mixture of 2:4-dinitrophenylhydrazones is applied to the column in 75 ml. ethyl acetate. The chromatogram is developed with ethyl acetate, ethanol, aqueous ethanol and dilute alcoholic sodium carbonate solution in that order. A pressure of 25 cm. Hg of CO_2 is used to speed up the rate of flow of the solvents. 15 ml. fractions are collected from the column and their yellow colour measured in a photoelectric absorptiometer using a 1 cm. cell and a Chance OB 1 blue light filter.

according to the particular 2:4-dinitrophenylhydrazones it is desired to investigate and the relative amounts present. Thus to effect a complete separation of the 2:4-dinitrophenylhydrazones of phenylpyruvic and *p*-hydroxyphenylpyruvic acids the amount of 95% ethanol passed through the column is increased; similarly if the separation of the dibasic ketoacid 2:4-dinitrophenylhydrazones is desired a less alkaline eluant (0·1 M-Na_2CO_3) may be used with advantage.

In applying the method to urine the following procedure is adopted. The urine is treated with an equal volume of 0·01 M 2:4-dinitrophenylhydrazine in 2 N-HCl and allowed to stand at room temperature for 24 hr. The 2:4-dinitrophenylhydrazones thus

Table 1

Solvent mixtures

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Ethyl acetate	1	1	1	–	–	–	–	–
95% ethanol	–	1	3	1	9	1	1	1
Water	–	–	–	–	1	1	3	–
0·5 M-Na_2CO_3	–	–	–	–	–	–	–	3
Total volume used in ml.	850	400	200	400	1000	400	300	600

Movement of 2:4-dinitrophenylhydrazones

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Acetone	+ + +	.	.	.	.	.	.	.
Acetaldehyde	+ + +	.	.	.	.	.	.	.
Benzaldehyde	+ + +	.	.	.	.	.	.	.
Phenylacetaldehyde	+ + +	.	.	.	.	.	.	.
Phenylpyruvic acid	–	–	–	+	+ + +	.	.	.
p-Hydroxyphenylpyruvic acid	–	–	–	±	+ +	.	.	.
Pyruvic acid	–	–	–	–	+	+ +	.	.
Oxalacetic acid	–	–	–	–	–	–	–	+ +
α-Ketoglutaric acid	–	–	–	–	–	–	–	+

The table indicates the solvent mixtures and volumes we have found useful and also the movements of the various 2:4-dinitrophenylhydrazones we have investigated.

It will be seen from the table that the neutral 2:4-dinitrophenylhydrazones are eluted immediately by ethyl acetate and do not interfere with the subsequent separation of those of the keto-acids.

Using suitable calibration curves made from standard solutions of the various 2:4-dinitrophenylhydrazones in the solvent in which they are eluted from the column, we have been able to estimate quantities of ketoacids of the order of 1 mg. with an error of less than 5%.

The volumes of the various eluants may be varied

formed may be extracted with ethyl acetate and applied to the column *in toto*, or may first be fractionated by suitable partition between ethyl acetate and M-Na_2CO_3 solution into neutral and acidic fractions. The final solution in ethyl acetate should be dried over Na_2SO_4 before being applied to the column. A suitable amount of urine for the size of column described is of the order of 1/10th of a 24 hr. specimen in the case of normal subjects and down to 1/200th of a 24 hr. specimen in certain pathological conditions.

Several different ketoacids have been found to occur regularly in normal human urine, these are being identified. The method is also being applied to the study of urine from cases of phenylketonuria.

Production and Utilization of Methylglyoxal by Tissue. By H. M. SALEM and E. M. CROOK. (*Department of Biochemistry, University College, London*)

Dakin & Dudley (1913) discovered enzyme glyoxalase which catalyses the reversible conversion of methylglyoxal into D-lactic acid in all animal tissues. They also found an inhibitor antiglyoxalase in the pancreas. Toenneissen & Fisher (1926) showed that hexose-diphosphate was converted to methylglyoxal when the latter was digested with muscles in the presence of antiglyoxalase to retard the action of glyoxalase on methylglyoxal.

Ariyama (1928) repeated these experiments but failed to show the influence of antiglyoxalase.

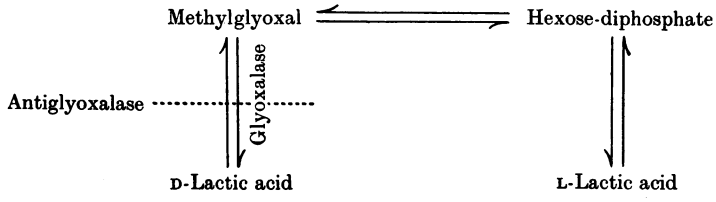
It has now been confirmed that when hexose-diphosphate is incubated with liver homogenate, methylglyoxal is produced. The methyl glyoxal formed was measured quantitatively by a method using glyoxalase.

Ariyama's suggestion that the enzyme glyoxalase was destroyed by incubation was disproved, and evidence was obtained to show that it was the glyoxalase coenzyme, glutathione, which was destroyed, thus resulting in the inactivation of glyoxalase. Evidence was also obtained to show that the reaction

Hexose-diphosphate ⇋ methylglyoxal

was reversible. When methylglyoxal was incubated with liver homogenate and antiglyoxalase which inhibited the destruction of methylglyoxal by glyoxalase, half of the total inorganic phosphorus and three quarters of the methylglyoxal disappeared. The disappearing inorganic phosphorus was found to accumulate in the hexose diphosphate and phosphopyruvic fractions. The latter might be derived from the first through glycolysis, and could itself give rise to lactic acid by further breakdown.

The following scheme would summarize the whole system:



The observation by Dakin & Dudley (1913) that a mixture of both D- and L-lactic acid was formed in unequal proportions when methylglyoxal was incubated with crude glyoxalase preparations could then be explained. Glyoxalase acting on methylglyoxal produced D-lactic acid. At the same time, some methylglyoxal was converted to hexose-diphosphate by the reversible reaction shown above and the latter was converted to L-lactic acid through glycolysis.

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The Mode of Action of Glyoxalase. By E. M. CROOK and KATHLEEN LAW. (*Department of Biochemistry, University College, London*)

Glyoxalase, in the presence of reduced glutathione ('GSH'), converts substituted glyoxals to the corresponding hydroxy acids.

$$R.CO.CHO \xrightarrow[H_2O]{} R.CHOH.COOH$$

(Dakin & Dudley, 1913).

By iodine titration of the —SH groups, Jowett & Quastel (1933) obtained evidence for the formation of a methylglyoxal —GSH compound in the absence of enzyme. Giršavičius (1935) showed that in the presence of glyoxalase there was a similar disappearance of free —SH groups, indicating the formation of some type of methylglyoxal —GSH complex.

The scheme of reaction postulated at that time was as follows:

$$CH_3.CO.CHO + GSH \rightleftharpoons CH_3.CO.CHOH.SG \xrightarrow[H_2O]{} CH_3.CHOH.COOH + GSH.$$

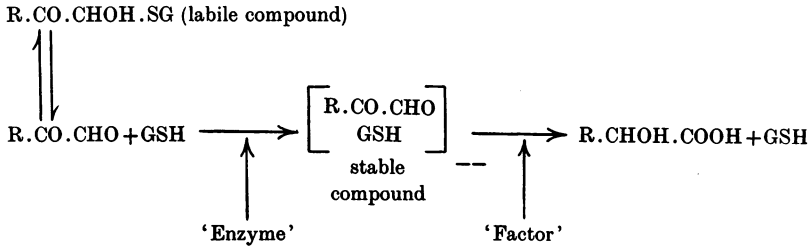
In 1936 Yamazoye showed that the compound formed from methylglyoxal and GSH in the presence of crude enzyme was different from that produced

when the substrates react in the absence of enzyme. The latter was labile, readily regenerating methylglyoxal and GSH; its —SH groups reacted when an excess of I_2 was added and it gave strong reactions for keto-groups. The compound formed in the presence of the enzyme was much more stable; it did not regenerate methylglyoxal and GSH but was slowly hydrolysed above pH 7 to GSH and lactic acid, and gave no reactions for keto-groups.

Hopkins & Morgan (1948) separated and purified two components of glyoxalase. They showed that the 'enzyme' acts on methylglyoxal and on phenylglyoxal at the same rate. The second component or 'factor' accelerates the conversion of methylglyoxal to lactic acid, but causes no increase in rate when phenylglyoxal is the substrate.

In this communication evidence will be given which shows that the formation of the stable glyoxal —GSH compound is brought about by the 'enzyme'. This enzymic compound under experimental conditions breaks down spontaneously into the corresponding hydroxy acid and GSH. The 'factor' accelerates the breakdown of the methylglyoxal —GSH compound, but has no action on the phenylglyoxal compound.

The reactions are shown to be:



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The Mechanism of the Anti-Aneurin Activity of Bracken (*Pteris aquilina*). By W. C. Evans, N. R. Jones and R. A. Evans. (*Biochemical Laboratories, Department of Agriculture, University College of Wales, Aberystwyth*)

It is now established that the inclusion of air-dried green bracken in the feed of rats (Weswig, Freed & Haag, 1946; Evans & Evans, 1949; Thomas & Walker, 1949) and horses (Roberts, Evans & Evans, 1949) produces an avitaminosis B_1 in these animals, although the diet as a whole is originally adequate in its aneurin content. In bovine bracken poisoning, evidence is accumulating (Hughes & Evans, unpublished) showing that when clinical symptoms are evident, there is also a state of B_1 deficiency, although this condition in the ruminant appears to be complicated by other factors.

The 'anti-aneurin factor' of bracken has now been shown to possess the following properties:

(1) It is very stable in the intact leaf after air drying at room temperature, and withstands heating at 100° C. for a considerable time in this condition. Steaming or autoclaving results in a rapid inactivation.

(2) De-proteinized water extracts, and ethanol concentrates of bracken, contain no demonstrable anti-aneurin activity.

(3) The factor is extracted from the leaf by chloroform—water at pH 7·5–8; 10 g. green leaf powder extracted in this way with 50 ml. solution overnight at 0° C. and filtered, gives a clear extract able to inactivate 5–10 μg. aneurin per ml. The anti-aneurin factor in this solution is thermolabile, and behaves like an enzyme. It can, however, be freeze-dried without loss of activity.

(4) By the use of *Neurospora crassa* mutant 9185, and *Phycomyces blakesleeanus* as indicators, it has been made highly probable that cleavage of the aneurin molecule occurs at the methylene bridge by the action of the bracken enzyme.

(5) This view of the chemical course of aneurin inactivation is supported by chromatographic evidence; the presence of the thiazole component as one of the products of reaction has been definitely established by this technique. Chemical isolation is in progress.

(6) A low temperature concentrate of the bracken enzyme (activity 100 μg. B_1/ml.) when added to a standard basal diet adequate for the growth of rats

has the same effect on the animals as the original bracken leaf powder diet, whilst a parallel experiment with a boiled concentrate added to the diet is quite innocuous and allows normal growth.

The existence of a plant thiaminase has not, as far as we are aware, been reported on before; there are indications that bracken is not the only plant possessing it, *Equisetum arvense* (horse-tail) for instance has been shown by us to contain it.

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The Avidity of Amino-acids for the Ions of Heavy Metals. I. Amino-acids with only two Ionizing Groups. By A. ALBERT. (*Department of Medical Chemistry, The Australian National University*)

It has long been known that various amino-acids form sparingly ionized complexes with the ions of heavy metals. So far, quantitative data has been available only for glycine (Flood & Loras, 1945). The discovery that pteridines (including folic acid) form similar complexes (Albert & Brown, 1949) made it desirable to obtain quantitative data for the amino-acids which would be important natural competitors. The discovery of comparable chelating properties in riboflavine and certain purines (Albert, unpublished) adds point to the investigation.

The following are the logarithms of the stability constants for the following equilibrium:

$$\frac{[\text{complex}]}{[\text{metal ion}]\,[\text{free anion}]^2}$$

(determined potentiometrically in 0·01 M aqueous solutions at 20°).

It is evident that each of those amino-acids that were tested with all eight metals has the same order of preference for metallic ions, copper always being the most firmly bound. There is no reason to expect any other amino-acid to depart from this order, provided it has only two ionizing groups. Proline complexes have the highest stability and asparagine complexes are among those that have the least. Nevertheless, at pH 7·3 asparagine can out-compete proline for securing metallic ions. This is because of the low basic pK_a of asparagine which ensures a high concentration of anions and hence (by the mass action law) a greater quantity of complex is formed. Hence the function $\bar{n}$ (Bjerrum, 1941), which represents the fraction (of the theoretically formable amount of complex) actually formed at a given pH, is of greater interest to biochemists than the stability constant, from which it can be derived.

	Cu^{++}	Ni^{++}	Zn^{++}	Co^{++}	Cd^{++}	Fe^{++}	Mn^{++}	Mg^{++}
Glycine	15·4	11·0	9·3	8·9	8·1	7·8	5·5	*c.* 4
Alanine	15·1	—	—	8·4	—	7·3	—	—
Valine	15·1	—	—	8·6	—	6·8	—	—
Phenylalanine	14·9	—	—	7·9	—	6·3	—	—
Serine	14·6	—	—	8·0	—	7·0	—	—
Methionine	14·7	—	—	7·9	—	6·7	—	—
Proline	16·8	11·3	10·2	9·3	8·7	8·3	*c.* 5·5	<4
Tryptophan	15·9	10·2	9·3	8·5	8·1	7·6	*c.* 5	<4
Asparagine	14·9	10·6	8·7	8·4	6·8	6·5	*c.* 4·5	*c.* 4

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Chemical Changes in the Developing Chick Embryo Related to the Deoxyribonucleic Acid Content of the Nucleus. By J. N. DAVIDSON, I. LESLIE, R. M. S. SMELLIE and R. Y. THOMSON. (*Biochemistry Department, The University, Glasgow*)

Deoxyribonucleic acid (DNA) has been found to be constant in amount in nuclei isolated from cells of different tissues of animals of a single species (Vendrely & Vendrely, 1948, 1949; Mirsky & Ris, 1949). Recently Davidson & Leslie (1950) have shown the value of this constant element of the cells in revealing chemical changes occurring in growing tissues. The composition of the predominant cell type is obtained when components of the tissues are expressed as amounts per unit of DNA. In this way it is possible to avoid some obscuring influences of variable tissue composition on results expressed in terms of wet or dry weights.

Table 1. *The deoxyribonucleic acid content of the nuclei of cells of different tissues of the fowl expressed as* $\mu g. \times 10^{-7}$ *deoxyribonucleic acid phosphorus (DNAP) per nucleus*

DNAP	Erythrocyte	Liver	Kidney	Spleen	Heart	Pancreas
By phosphorus determination	2·49	2·56	2·20	2·54	2·45	2·61
By diphenylamine test	2·43	2·33	2·23	2·40	2·45	2·32
By ultra-violet absorption	2·49	2·52	2·37	2·44	2·53	2·60

Table 2. *Growth rates* ($k = \tan \alpha$) *relative to growth rate of DNAP per organ*

Organ	Incubation period (days)	Acid-soluble phosphorus	LP	RNAP	PN
Brain: (1)	8–14	2·07	2·28	1·95	2·04
(2)	15–20	1·28	1·61	1·27	1·31
Heart	8–20	0·99	0·99	0·98	1·04
Liver: (1)	8–14	1·09	1·26	1·07	1·23
(2)	15–20	1·06	1·02	0·94	1·06

Preliminary results (Table 1) have confirmed that the amount of DNA is constant per nucleus in cells from a number of different organs of the adult fowl. When the protein nitrogen (PN), lipid phosphorus (LP), and ribonucleic acid phosphorus (RNAP) of the embryonic chick brain, heart, liver, and skeletal muscle are expressed as amounts per unit of deoxyribonucleic acid phosphorus (DNAP), i.e. as amounts per cell, and are plotted against incubation age, the following information is obtained:

(1) In brain, liver and skeletal muscle, PN increases per cell from the 8th to 18th day of embryonic development. In heart it remains more or less constant.

(2) Prior to hatching PN per cell decreases in all four tissues. The cell content of PN rises sharply to nearly twice its embryonic level 2–3 days after hatching.

(3) Brain is characterized by the relatively large increase of LP and RNAP per cell throughout embryonic growth and after hatching. Only in embryonic heart cells do LP and RNAP remain constant or decrease slightly during the period investigated.

These rising amounts of cellular components in brain, liver, and muscle are believed to reflect an aspect of the differentiation of these tissues during their development (Davidson & Leslie, 1950).

The growth rates of the various components have been compared with the growth rate of DNAP per organ in brain, heart, and liver (cf. Teissier, 1931; Needham, 1934). These are shown in Table 2 where the results are based on a larger number of determinations than those published previously (Davidson & Leslie, 1950). In brain the relative growth rates for PN, LP, and RNAP are characteristically high, suggesting that growth in this tissue involves a greater increase in cell size than occurs in other tissues. The discontinuities in the relative growth rates of components of brain and liver at 14 to 15 days confirm earlier observations of a critical phase in embryonic development at this stage (Needham, 1931).

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The Thermodynamics of the Active State of Muscle. By A. V. HILL. (*Biophysics Research Unit, University College, London*)

If a quick stretch is applied to a muscle shortly after a single shock the resistance to stretch is found to rise rapidly from the middle of the latent period onwards, i.e. before any shortening is detected in an ordinary contraction. The state of enhanced rigidity made evident in this way begins at about the same moment as the heat production and as the increase of optical transparency described by D. K. Hill (1949). It reaches its full extent very early, soon after the latent period is over; it is maintained for a time and then gradually disappears in 'relaxation'.

Stretched during contraction a muscle resists strongly, with a force substantially greater than that developed isometrically. The force diminishes sharply directly the stretch ends. The work (W) done in stretching it and the heat (H) produced are measured. If the stretch is timed to occur during the earlier phase of contraction (as distinguished from relaxation) ($H - W$) is considerably less than the heat which the muscle would have produced had it not been stretched. The stretch is applied between lengths at which the tension at rest is negligible, so that none of the work remains as elastic potential energy at the time when the total heat is measured after relaxation. A substantial part, therefore, of the work is absorbed. If the stretch is applied later, so as to fall partly during the phase of relaxation, the absorption of work is less. If applied wholly during relaxation, all the work appears as extra heat. The power of absorbing work, like that of doing work, is clearly a property of the active state. If activity is maintained by repeated stimuli the whole of the work done in stretching a muscle can be absorbed.

It is difficult not to suppose that the work which disappears is used in driving some chemical process backwards, presumably the same process, reversed, as supplies work when a muscle normally shortens. There is positive heat production associated with shortening as such and negative heat of lengthening. In the equation, therefore (A. V. Hill, 1949),

$$E = A + ax + \int P dx$$

(energy = activation heat + shortening heat + work),

the sign of x may be either positive or negative. The active state appears to involve a physical framework mechanically coupled with reversible chemical reactions.

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Volatile Fatty Acids in *Ascaris lumbricoides*. By E. BALDWIN and VIVIEN MOYLE. (*Biochemical Department, University of Cambridge*)

DEMONSTRATIONS

Fractionation of Hydrolysis Products of Amylose by Electrokinetic Ultrafiltration in an Agar-agar Jelly. By R. L. M. SYNGE (*The Rowett Research Institute, Bucksburn, Aberdeenshire*) and A. TISELIUS (*Institute of Biochemistry, Uppsala*)

It should be possible to effect separations depending on differences in the rates of ultrafiltration of solutes by using an indefinitely extended ultrafilter such as a jelly. Experiments using pressure to force solvent through jellies resulted in their mechanical breakdown. It was therefore proposed to use the electrokinetic flow due to a potential gradient; here, frictional forces due to flow are throughout the gel opposed by electrically produced forces. The authors and Dr A. J. P. Martin have frequently considered these possibilities (cf. *Discussions Faraday Soc.* no. 7 (1949) 'Chromatographic Analysis').

The products of hydrolysis of amylose seemed promising test substances, since they are (Swanson & Cori, 1948) a randomly produced mixture of chain molecules, whose iodine colours are correlated with chain length (Swanson, 1948). An amylose fraction from potato starch (Bourne, Donnison, Peat & Whelan, 1949) was hydrolysed with 10N-HCl for 20 min. at 20°; the HCl was evaporated *in vacuo* and the neutralized product was incorporated as inlay in the apparatus of Consden, Gordon & Martin (1946, method B) using final concentrations: sodium acetate 0·02N; acetic acid 0·02N; iodine (dissolved with KI) 0·001N; agar-agar (cf. Gordon, Keil & Šebesta, 1949) 1% (w/v); hydrolysed amylose (inlay only) 0·2% (w/v). With 4 V./cm. separation of differently coloured components of the mixture was observed,

yellow migrating fastest, followed by orange, red, pink, lavender and blue, all with the electroendosmotic stream towards the cathode. Some blue material, perhaps phosphorylated, migrated anodically. Without hydrolysis of the amylose no migration or diffusion whatever occurred. Prolonging the hydrolysis diminished the slower blue-pink components relative to those staining red-orange.

The hydrolysed amylose was also investigated in the standard electrophoresis apparatus, in absence of agar, using the same buffer medium. Even after prolonged electrophoresis (1072 min. at 2·87 V./cm., +0·6° C.) only two boundaries were observed. The main component showed no mobility and no sign of inhomogeneity. The second component moved quite fast towards the anode (mobility 17·4 $cm.^2 sec.^{-1} V.^{-1}$) and is presumably identical with the anodic material of the agar experiment.

The separation in agar could reasonably be attributed to a molecular-sieve mechanism. The considerable sensitivity to agar concentration of the relative rates of the components is suggestive of this. With adsorption also occurring and following Traube's Rule, separation will be assisted. In ordinary, grossly particulate chromatograms, molecular-sieve action within the granules counteracts the operation of Traube's Rule. Electrokinetic ultrafiltration may therefore prove generally useful for separating substances chemically similar but differing in molecular weight.

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Apparatus for Mass-Production Two-Way Paper Chromatography. By S. P. Datta, C. E. Dent and H. Harris. (*Department of Biochemistry, University College, London, and University College Hospital, London*)

The principle involved here enables large numbers of two-way (two-dimensional) chromatograms to be run simultaneously. An adaptation for 12 paper squares 20 × 20 cm. is shown in Fig. 1 and in the demonstration. The solution to be analysed is placed near the corner of the paper as usual, and the papers threaded on the four rods of the frame. Collars of 2 cm. length are also threaded between the papers so as to keep them from touching each other. The frame and papers are placed in a dish containing the first solvent and the whole enclosed in any suitable box. The solvent soaks upwards until it reaches the far edge. The papers, still held on the frame, are dried, and then run with their other edge downward in the second solvent. They are again dried in the frame.

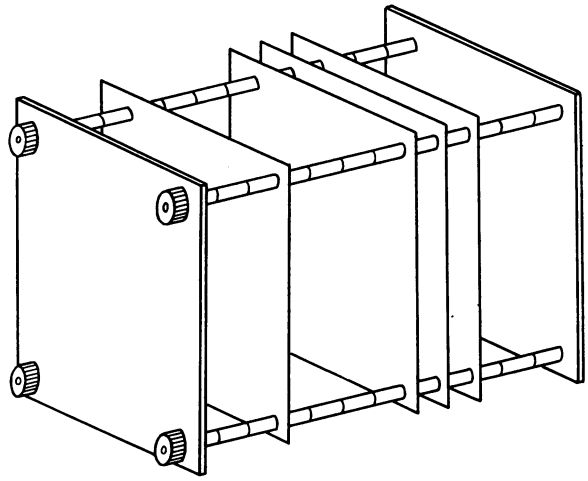

Fig. 1.

We find the apparatus valuable for routine urine testing. The resolving power for the amino acid spots is not quite as good as with the larger chromatograms (18 × 22 in. or 30 × 30 in.). The frame is made of duralumin. It has been found to be satisfactory for use with phenol and collidine-lutidine as solvents.

The Thermodynamics of the Active State of Muscle. Equipment and Methods. By A. V. Hill. (*Biophysics Research Unit, University College, London*)