PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **288th Meeting** *of the Biochemical Society was held in the Department of Biochemistry, University New Buildings, Teviot Place, Edinburgh, on Friday,* 21 *July* 1950, *when the following papers were read:*

COMMUNICATIONS

A 'De-branching' Enzyme in Bean and Potato.

By P. N. Hobson, W. J. Whelan and S. Peat.
(*Department of Chemistry, University College of North Wales, Bangor*)

In the course of an investigation of the Q-enzyme (Barker, Bourne, Wilkinson & Peat, 1950) of the broad bean we attempted to remove α-amylase impurity by absorbing it on starch grains from an aqueous ethanol solution of a Q-preparation (cf. Holmberg, 1933). Not only was α-amylase removed, but so also was Q-enzyme. The treated solution was without action on pure amylose, but it contained an enzyme (R-enzyme) having the novel properties of causing substantial increases in the iodine-staining powers (blue value) and limits of convertibility to maltose by β-amylase of both amylopectin and its β-amylase limit dextrin (β-dextrin). These reactions are not influenced by the addition of mineral phosphate. A summary of the properties of the new enzyme is given in Table 1.

Table 1. *Properties of R-enzyme*

	Action of R-enzyme	
Change in:	On β-dextrin	On amylopectin
Blue value	0·140→ 0·265	0·160→ 0·250
β-Amylolysis limit (%)	2·8 →61·6	48·4 →66·0
Reducing power (as % maltose)	0 →11·4	0 → 1·0
Viscosity (% fall)	85	51

The enzyme has been isolated as a stable powder from the broad bean and the potato. When it acted on whole potato starch or on amylose containing amylopectin the observed changes were in direct proportion to the amylopectin contents of the substrates, an indication that it is the branched, and not the linear, structures which are attacked. The increase in blue value which accompanies this 'de-branching' action would suggest that synthesis of 1:4 links is also taking place, with the formation of an amylose-type polysaccharide. It may be, however, that the action of R-enzyme is confined to the hydrolysis of 1:6 links and that the rise in blue value is due to a difference between the iodine-staining capacity of branched and unbranched chains. The products of R-enzyme action on β-dextrin or amylopectin are (*a*) not attacked by Q-enzyme, (*b*) of greater reducing power and lower viscosity than the untreated polysaccharides, and (*c*) have much lower blue values than amylose. These facts indicate that R-enzyme does not synthesize chain-forming (i.e. 1:4) links.

The simultaneous action of Q- and R-enzymes on amylose yields a product closely resembling an R-enzyme-treated amylopectin. R-enzyme has no action on unbranched chains of any length between that of amylose (approx. 200 units) and achroic dextrins (approx. 9 units), and is without influence on the synthesis of amylose by phosphorylase. These properties suggest that the de-branching action of R-enzyme is irreversible. In this case, the failure to achieve complete β-amylolysis of R-enzyme-treated β-dextrin and amylopectin is due either to the presence of two types of branch link, only one of which is hydrolysed by R-enzyme, or to a low affinity of β-amylase for the very short dextrin chains. The enzyme does not attack the 1:6 links in isomaltose or bacterial dextran.

An enzyme with a similar function has been isolated from muscle by Cori & Larner (1950).

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Cori, G. T. & Larner, J. (1950). *Fed. Proc.* **9**, 163.

Holmberg, O. (1933). *Biochem. Z.* **258**, 134.

The Role of Z-Enzyme in β-Amylolysis. By GWEN J. THOMAS, W. J. WHELAN and S. PEAT. (*Department of Chemistry, University College of North Wales, Bangor*)

In 1949, Peat, Whelan & Pirt reported that crystalline sweet potato and specially purified soya bean β-amylases effected a limiting conversion of amylose to maltose of only 70 % instead of the almost complete conversion normally found with β-amylase preparations; a limit dextrin of high blue value (B.V.) remained. It was shown that the presence of a second enzyme (Z-factor) was necessary before complete β-amylolysis could occur.

The Z-factor has now been isolated as a stable solid by dissolving a standard β-amylase preparation from soya bean in 0·2M-acetate buffer, pH 3·0, heating for 1·5 hr. at 35°, adjusting the pH to 4·8 and freeze-drying. It is completely free from β-amylase and contains only a trace of α-amylase, the action of which is entirely inhibited if digestion is carried out at pH 4·8. The action of pure Z-enzyme on amylose and amylopectin is compared in Table 1 with that of α-amylase.

It is clear that although a mixture of α- and pure β-amylases acting on amylose simulates the combined action of pure β-amylase and Z-factor, the actions of the same enzyme mixtures on amylopectin are distinct enough to prove that Z-enzyme and α-amylase are not identical. Z-factor appears to eliminate some structure in the amylose molecule which forms a barrier to the action of pure β-amylase. This impeding structure is not a normal (1:6) branch link and is not contained in the 'outer' chains of amylopectin. Presumably, α-amylase circumvents the obstruction in amylose in a manner similar to that by which it by-passes the branch linkage in amylopectin, making both polyglucoses susceptible to further hydrolysis by β-amylase. When amylose is digested with Z-factor, the enzyme destroyed and pure β-amylase added the resulting conversion to maltose is 97·7 %, proving that the action of Z-factor is not dependent upon the presence of β-amylase. When, in this experiment, α-amylase replaces Z-factor the limiting conversion to maltose is less complete, being 89·4 %, a difference to be expected if α-amylase does not remove, but merely by-passes the obstruction which is eliminated by Z-enzyme; some parts of the starch chains would still be protected from the end-wise attack of β-amylase.

Table 1. *Action of enzymes on amylose and amylopectin*

(All digests incubated at 35·0° and pH 4·8. % conv. = % conversion to maltose; A.V. = absorption value (680 mμ.) of iodine-stained residual polysaccharide; β = crystalline sweet potato β-amylase; Z = purified Z-factor; α = freeze-dried salivary α-amylase; stock soya = stock soya bean β-amylase (containing Z-enzyme).)

	Amylose, B.V. 1·45			
	6 hr.		19 hr.	
Enzyme(s)	% conv.	A.V.	% conv.	A.V.
Z	0·0	1·40	1·3	1·39
α	1·8	1·37	—	—
β	67·3	0·357	67·5	0·340
β+Z	90·5	0·079	93·4	0·038
β+α	96·0	0·010	—	—
Stock soya	91·5	0·055	92·0	0·042

	Amylopectin, B.V. 0·158			
	2 hr.		7 hr.	
Enzyme(s)	% conv.	A.V.	% conv.	A.V.
Z	0·0	0·158	0·0	0·160
α	3·8	0·106	7·3	0·084
β	51·2	0·088	51·5	0·088
β+Z	52·3	0·088	51·5	0·088
β+α	58·3	0·032	64·9	0·023
Stock soya	50·9	0·095	52·5	0·073

Table 1 shows that Z-enzyme *per se* is without action on the 1:4-glycosidic links of either amylose or amylopectin, and that it cannot, therefore, be an amylase. It may be a phosphatase; the purified enzyme possesses powerful phosphatase activity with respect to glycerophosphate. On the other hand, the ester phosphate content of amylose is too low to admit of there being present even one phosphate group per amylose chain. Further experiments are in progress.

REFERENCE

Peat, S., Whelan, W. J. & Pirt, S. J. (1949). *Nature, Lond.*, **164**, 499.

The Determination of Reducing Sugar in Blood. By W. N. M. RAMSAY. (*Department of Biochemistry, University of Edinburgh*)

Reducing sugar can be determined in a $Cd(OH)_2$-$BaCO_3$ filtrate of whole blood by ferricyanide oxidation and photometric estimation of the resultant ferrocyanide after conversion to the pink ferrous-dipyridyl complex ion. As a routine procedure, duplicate analyses are made on a single specimen of 0·1 ml., but the sensitivity of the method permits scaling down so that a single analysis can be done on 0·02 ml. blood. The method is a little simpler and more rapid than the common volumetric procedures, and has the additional advantage that the final coloured product is a well defined stable substance.

The author wishes to thank Mr D. Purves and Miss D. C. Fairweather for their assistance.

The Configuration of the Anomeric Carbon Atoms in some Cardiac Glycosides. By W. KLYNE. (*Postgraduate Medical School, London, W.* 12)

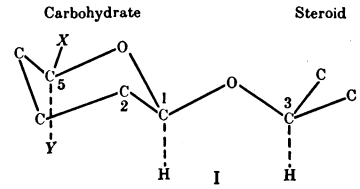
The method of molecular rotation differences (Barton, 1945) has been applied to the steroid glycosides. It can be used to indicate whether such compounds are α- or β-glycosides. All the cardiac glycosides to which the method can be applied probably have the same stereochemical type of union between steroid and carbohydrate.

Klyne (1950) has shown that the method of molecular rotation differences can be applied to structural problems concerning the cardiac aglycones and toad poisons. It has now been shown that in the steroid glycosides the rotation contribution of the carbohydrate component (ΔC) is almost independent of the nature of the steroid component:

Molecular rotation $= [M]_D = [\alpha]_D \times$ mol.wt./100,
$\Delta C = [M]_D$ of steroid glycoside $- [M]_D$ of free steroid.

The rotations of a series of synthetic steroid 3-glycopyranosides suggest the following general rule, namely, that the carbohydrate contribution ΔC is very approximately equal to $[M]_D$ of the corresponding α- or β-methylglycopyranoside.

A few compounds deviate considerably from this rule, probably because of unusual consitutional factors or solvent differences, e.g. the cholestanyl-glucosides of Linstead (1940). The rotations of these compounds were determined in pyridine, and some ΔC values calculated from them do not agree well with the $[M]_D$ values of the methylglucopyranosides in water and of their acetates in chloroform. However, the differences between the rotations of the anomeric pairs of cholestanylglucosides are all of the expected sign and order of magnitude. Apart from these exceptions the differences between ΔC and $[M]_D$ of the appropriate methylglucosides are between $+100$ and $-70°$ (mean difference 38° for a series of twenty-six compounds). These differences are much smaller than the differences in $[M]_D$ between anomeric α- and β-methylglycosides (350–500°).



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The method of rotation differences may therefore be used to indicate the configuration at the anomeric carbon atom in naturally occurring steroid glycosides, if $[M]_D$ values for the corresponding methylglycosides are known. Table 1 shows the conclusions reached for a number of cardiac glycosides. It seems very probable from the conditions in which these com-

Table 1. *Probable configuration of the anomeric carbon atoms in some cardiac glycosides*

Carbohydrate	$[M]_D$ of methylglycopyranosides: α	$[M]_D$ of methylglycopyranosides: β	Rotation contribution of carbohydrate in cardiac glycosides (ΔC)	Configuration suggested for anomeric C-atom
D-Cymarose	+370	(+ 40)*	−44, −22, −6, +22, +39	β
D-Diginose	+142	(−200)	−97, −156	β
D-Digitalose	+240	(−100)	−24, −44	β
D-Lyxose	+ 98	(−240)	−163	β
D-Sarmentose	+275	− 69	−119, −151	β
L-Rhamnose	−111	+170	−170, −178, −211	α
L-Thevetose†	(−260)	(+110)	−338	α†

* Values in parentheses are calculated from $[M]_D$ values of other derivatives.
† The natural acetyl L-thevetosides must have the same configuration.

pounds are hydrolysed that they are pyranosides and not furanosides. Comparison of the ΔC values of these glycosides with the rotations of the corresponding α- and β-methylglycopyranosides indicates that the D-glycosides are β and the L-glycosides α. It appears, therefore, that in all these compounds the stereochemistry of the union between carbohydrate and steroid is the same (I. For the β-D-lyxoside, $X = Y = H$; for the other β-D-compounds $X = CH_3$; $Y = H$; for α-L-compounds, $X = H$; $Y = CH_3$.)

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Klyne, W. (1950). *Biochem. J.* **46**, xxii.
Linstead, R. P. (1940). *J. Amer. chem. Soc.* **62**, 1766.

Studies on the Inhibition of Alkaline Phosphatase. By E. DICZFALUSY, B. ALDMAN, B. HÖGBERG and T. ROSENBERG (introduced by G. F. MARRIAN). (*Department of Women's Diseases, Karolinska Sjukhuset, Stockholm*)

The action of various inhibitors of the non-competitive type on the alkaline kidney phosphatase has been investigated. These inhibitors include the oestrogenic hormones, some of their derivatives as the phosphates, the glucoside, etc., and some synthetic compounds. Particularly strong inhibition has been found with oestradiol-3:17-diphosphate. The dependence of the inhibition on the pH has been demonstrated. The nature and mechanism of the inhibition and some biological relations were discussed.

Evidence for Transfructosidation in the Jerusalem Artichoke. By J. EDELMAN and J. S. D. BACON. (*Department of Biochemistry, University of Sheffield*)

When examined by paper partition chromatography the carbohydrates in extracts of the artichoke tuber appear as a series of ketose-containing spots; the lowest spot (1) has an R_F equal to that of sucrose, the highest (n) resembles inulin in having an R_F of zero (Bacon & Edelman, 1949). If aqueous extracts (pH 6·5) are incubated at 30° little change is noticed in the ketose content of the lower spots, but free fructose appears in measurable amounts (Edelman & Bacon, 1949).

When 1·5% sucrose was added and the extract incubated at 28° for 15 hr. the following changes were observed:

(1) Fructose was liberated, but in no greater amounts than in the same extract without added sucrose (fructose increased from 33 to 79 μg./20 μl.).

(2) The ketose content of spot 1 decreased markedly (from 299 to 252 μg./20 μl.).

(3) The ketose content of spots 3 to n decreased also (from 1219 to 1128 μg./20 μl.).

(4) The ketose content of spot 2 increased (from 143 to 236 μg./20 μl.).

No such changes were observed with boiled extracts; the reaction had a pH optimum of 6·0–6·5.

These results are most simply explained by assuming the transference of fructofuranoside residues from material of low R_F to sucrose, to give a trisaccharide (spot 2). This hypothesis was supported by a study of the action of dialysed carbohydrate-free enzyme preparations on mixtures of inulin and sucrose. Under these conditions spot 2, spot 3, and traces of higher spots appeared; the limiting velocity of the reaction was not reached in mixtures either with 5% sucrose (0·15M) or with 2% inulin (?0·004M). No effect of added inorganic phosphate could be demonstrated.

The extracts differ from levansucrase (Hestrin & Avineri-Shapiro, 1944) in showing little or no production of spot 2, or of higher spots, in the presence of sucrose alone, and glucose liberation does not appear to be a necessary part of the reaction, insignificant amounts appearing in tuber extracts, even on prolonged incubation with sucrose.

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Bacon, J. S. D. & Edelman, J. (1949). *Biochem. J.* **45**, xxviii.
Edelman, J. & Bacon, J. S. D. (1949). *Biochem. J.* **45**, xxix.
Hestrin, S. & Avineri-Shapiro, S. (1944). *Biochem. J.* **38**, 2.

The Influence of Sex on the Storage of Vitamin A. By V. H. Booth. (*Dunn Nutritional Laboratory, University of Cambridge, and Medical Research Council*)

When rats on a diet free of vitamin A were given vitamin A in any form (carrots, green leaves, halibut liver oil) more vitamin A was found in the livers of females than of males. This fact, though referred to in the literature, does not appear to have been studied. Males grew faster than females and lost their liver reserves of vitamin A faster. Hence the longer the interval between the beginning of dosing and the liver assay, the greater the sexual discrepancy. A difference was found, however, even when livers were assayed soon after a single dose. Since there had not been time for much growth, the storage difference could not be explained through the difference in growth rates. Moreover, in a group of rats of one sex given the same dose, although individual storage and individual growth both varied beyond the accuracy of measurement, there was no correlation between liver storage and growth rate: this applied to several groups of each sex.

Male rats are larger than females and therefore may have less 'surplus' for storage. However, the variation in storage by individual males of the same age and receiving the same dose showed no correlation with body weight. Nor was there a correlation among females. Further, young males from litters of twelve, though smaller than their female cousins from litters restricted at birth to two, still stored less than those females when given the same dose of halibut liver oil. Thus different body sizes of the sexes offers no explanation of the different capacities for accumulating vitamin A in livers.

The conclusion is reached that the difference between liver storage is not consequential to different growth rate or body size, but is a true sexual dimorphism. Although female rats lost their liver stores more slowly than males, females on a vitamin A-deficient diet barely outlived their brothers. An alternative storage site might explain this anomaly. The only significant reserves of the vitamin, elsewhere than in the liver, appear to be in the kidney (Johnson & Baumann, 1947) in which organ Moore & Sharman (1950), and also the author have found more vitamin in males than in females. The difference, however, is small and insufficient to counterbalance the superiority of female liver stores, but it is interesting through being in the opposite sense to differential liver storage.

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Johnson, R. M. & Baumann, C. A. (1947). *Arch. Biochem.* **14**, 361.

Moore, T. & Sharman, I. M. (1950). *Biochem. J.* **47**, xliii.

Vitamin A in the Kidneys of Male and Female Rats. By T. Moore and I. M. Sharman. (*Dunn Nutritional Laboratory, University of Cambridge, and Medical Research Council*)

Eden & Moore (1950) recently confirmed the observations of Johnson & Baumann (1947*a*,*b*) that when rats were given low doses of vitamin A, of up to about 30 i.u. daily, the concentration of vitamin A is higher in the kidney than in the liver. In preliminary experiments, moreover, they found that in male rats the kidneys contained more vitamin A than in females. This finding was in contrast with the general experience of Booth (1949), and others, that the livers of female rats tend to contain more vitamin A than those of males.

The results of further experiments on the distribution of vitamin A according to sex are given in Table 1. Young piebald rats were kept on a diet

Table 1. *Mean vitamin A in the kidneys and livers of rats receiving graded doses of vitamin A acetate for* 24–36 *days*

Vit. A, daily (i.u.)	Days of dosing	No. of rats of each sex	Kidneys				Livers			
			Males		Females		Males		Females	
			Total i.u.	i.u./g.	Total i.u.	i.u./g.	Total i.u.	i.u./g.	Total i.u.	i.u./g.
0	34	3	(0 9)	(0·8)	(0·8)	(0·7)	(7·1)	(1·8)	(1·7)	(0·4)*
10	36	3	3·4	2·0	3·3	2·3	8·4	0·4	2·5	0·4
20	35	3	16·4	8·8	10·0	8·1	6·9	0·6	30·3	5·2
40	26–33	7	41·0	24·1	6·4	4·9	88·5	9·4	160	24·4
80	24	4	29·2	18·6	5·1	4·3	396	45	720	115

* Maximum values, no blue colour observed with $SbCl_3$.

deficient in vitamin A, but adequate in vitamin E, for about 5 weeks, and were dosed daily with various levels of vitamin A acetate. It will be seen that after dosing with 10 i.u. of vitamin A daily the mean concentration of vitamin A was about the same in each sex, and exceeded the minute concentration in the liver. After doses of 20 i.u. the concentrations in the kidneys were somewhat higher and about the same in each sex, but the livers of the females contained much more vitamin A than those of the males. With 40 i.u. the concentration in the male kidneys was again raised without a corresponding rise in the female kidneys; the concentration in the males was thus about five times greater than in the females. At this level of dosing the concentration of vitamin A in the female liver was more than twice that in the male. With doses of 80 i.u. the concentrations in the kidneys were not further raised in either sex; the increased reserves in the liver still showed about the same ratio between the sexes. With one exception the levels of vitamin A in the pooled blood plasma of each group were greater in males than females, being 37 i.u./100 ml. at doses of 10 i.u., 56 at 20 i.u., 93 at 40 i.u. and 106 at 80 i.u. for males as against 31, 57, 79 and 73 i.u. for females.

Johnson & Baumann have observed that the concentration of vitamin A in the kidneys is usually high in rapidly growing animals. It remains to be decided, therefore, whether the high concentration in the male kidneys is directly due to sex, or indirectly to the well known superiority of the male over the female in growth rate. In continuing our experiments, trials are being made of the effect of sex hormones on the distribution of vitamin A.

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A Method for the Estimation of Small Quantities of Azide. By H. Lees. (*Department of Biological Chemistry, Aberdeen University*)

During the course of experiments on the oxidation of nitrate in soil it was noticed that azide, which was being used as a metabolic inhibitor, interfered with the estimation of nitrite by the Griess-Ilosva reagent. This reagent consists of two components: (1) sulphanilic acid which forms a diazo compound with the nitrite to be estimated, (2) α-naphthylamine which couples with this diazo compound to form a red dye. The intensity of the red colour formed by the overall reaction is therefore related to the initial concentration of nitrite and can be used to estimate it.

It was found that azide will react with the diazo compound and thus reduce the colour intensity produced on subsequent coupling with α-naphthylamine. The reaction is probably that referred to by Sidgwick (1937), in which an aryl azide results from the interaction of an aromatic diazonium compound and hydrazoic acid. The reaction can be made stoichiometric, and the reduction of final colour intensity caused by the addition of azide to a known amount of diazotized nitrite, prior to coupling with α-naphthylamine, can thus be used to estimate the amount of azide added. If a Hilger Biochem Absorptiometer fitted with a 4 cm. 25 ml. cell and an O.G. 1 filter is used, quantities of azide ranging from 0·1 to 0·3 μmol. may be conveniently estimated.

Trials have shown that azide added to fresh brain homogenates, to give final concentrations of 1×10^{-3} to 3×10^{-3} M azide, could be estimated in 0·1 ml. of the filtrate obtained after acid coagulation of the homogenate. The results given in Table 1 are typical.

Table 1. *Azide in* 0·1 *ml. filtrate* (μmol.)

Calculated from amount added to homogenate	Observed
0·33	0·34
0·26	0·27
0·20	0·20
0·13	0·13

The author hopes to use this estimation for investigating the precise action of azide on metabolic processes.

REFERENCE

Sidgwick, N. V. (1937). *The Organic Chemistry of Nitrogen*, p. 366. Oxford.

The Response in Urinary 'Reducing Steroid' Excretion to Stimulation of the Adrenal Cortex by Adreno-corticotropic Hormone.

By B. W. L. Brooksbank, Gwen M. Owen and F. T. G. Prunty.
(*Department of Chemical Pathology, St Thomas's Hospital Medical School, London, S.E.* 1)

A preliminary investigation into the response to adreno-corticotropic hormone (ACTH) of 'reducing steroid' excretion has been carried out with the object of assessing the relative value of different techniques used in its assessment.

Urines have been extracted by hand at pH 1 immediately after completion of the 24 hr. collection. Extraction was completed in 2 hr. Sufficient urine was extracted to enable three methods of analysis to be employed on each sample.

(*a*) *Molybdate reduction* was performed on crude neutral extracts (Heard & Sobel, 1946; Heard, Sobel & Venning, 1946). The use of phosphomolybdic acid made with molybdic acid A.R. (Hopkin and Williams Ltd.) or from ammonium molybdate with sulphuric acid gave weak and unstable colours. The reagent was therefore prepared from molybdic anhydride (Harrington Bros. Ltd.) and gave $E_{1\,cm.}$ (using Ilford filter 608) 0·39 for 85·2 μg. deoxycorticosterone heated for 1 hr.

(*b*) *Formaldehyde estimation* was performed with periodate oxidation of fractions soluble in water following benzene : water partition (Daughaday, Jaffe & Williams, 1948). The residues were dissolved in 0·5 or 1 ml. purified ethanol before oxidation. Conditions for the maximum recovery of formaldehyde by distillation were carefully defined, this being in the range 88–95%. After correction for these losses deoxycorticosterone gave 100% theoretical colorimetric values. $E_{1\,cm.}$ using Ilford filter 606 was found to be 0·46 for 115 μg. deoxycorticosterone.

(*c*) *Copper reduction* was performed on the ketonic fractions, by a micro-adaptation of the method of Talbot, Saltzman, Wixom & Wolfe (1945). The method is sensitive over the range 6–90 μg. deoxycorticosterone. Interfering opalescence of the final solutions was reduced by the aid of ether extraction prior to measurement.

Table 1 shows some typical results obtained with several subjects.

Table 1. *Increase in urinary 'reducing steroid' after ACTH*

(i) = Basal level. (ii) = Maximum figure after ACTH.

	ACTH		Deoxycorticosterone equivalent (mg./day)								
			Molybdate (*a*)		Formaldehyde (*b*)		Copper (*c*)		Increase (mg./day)		
Subject	mg./day	Days	(i)	(ii)	(i)	(ii)	(i)	(ii)	(*a*)	(*b*)	(*c*)
F	50	2	2·1	6·5	0·45	3·63	0·50	1·89	4·4	3·18	1·39
	50	2	2·2	8·3	0·42	5·53	0·45	2·80	6·1	5·11	2·35
N	50	2	3·1	4·5	0·44	1·80	0·46	1·24	1·4	1·36	0·78
	50*	2	2·3	5·5	1·11	3·96	0·54	1·49	3·2	2·85	0·95
H	50	2	—	—	0·86	2·46	0·52	1·39	—	1·60	0·87
S	40	10	3·3	5·4	0·79	2·26	0·59	1·76	2·1	1·47	1·17
	50†	10	3·9	9·6	0·79	6·26	0·59	3·70	5·7	5·47	3·11
Pe	25	7	—	—	—	—	0·65	0·61	—	—	—
	25‡	7	—	—	—	—	0·40	0·69	—	—	0·29
Pa	25	7	—	—	—	—	0·70	0·78	—	—	0·08

* After 7-day interval. † After 10-day interval. ‡ After 21-day interval.

The results show comparable increases by methods (*a*) and (*b*) which are consistently greater than those by method (*c*), indicating the possible excretion of appreciable amounts of material with C-17-glycol side chains. Examination of some benzene fractions in method (*b*) shows that the differences between the increases obtained by methods (*a*) and (*b*) may be largely accounted for by the material in this phase. At the basal levels methods (*b*) and (*c*) give approximately the same figures, indicating the absence of non-ketonic substances in the water-soluble material.

17-Ketosteroid excretion was followed in parallel with these studies and was found to exhibit less consistent response to ACTH administration than did that of corticosteroid-like substances.

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Some Differences Between the Metabolism of 2-Naphthylamine and of 2-Acetamidonaphthalene in the Dog and Cat. By D. B. CLAYSON. (*Department of Experimental Pathology and Cancer Research, School of Medicine, University of Leeds*)

Wiley (1938) isolated 2-amino-1-naphthylsulphuric acid from the urine of dogs fed with 2-naphthylamine; Dobriner, Hofmann & Rhoads (1941) found 6-acetamido-2-naphthol to be a metabolite of 2-naphthylamine in the rat, rabbit and monkey.

2-Amino-1-naphthol cannot readily be diazotized, and therefore it was necessary to develop a method for its quantitative estimation. The method adopted depends on the light adsorption of a benzene solution of the purple pigment formed by aeration of an ammoniacal solution of the aminonaphthol. 50–500 μg. of the compound can be estimated to an accuracy of $\pm 5\%$ using the 1 cm. cells on the Hilger Spekker absorptiometer. 6-Acetamido-2-naphthol is easily diazotized after hydrolysis, and the total diazotizable amine was determined by coupling with *N*-sulphato-ethyl-*m*-toluidine.

Our experiments have shown that 30–70 % of the 2-naphthylamine administered to the dog is excreted as conjugates of 2-amino-1-naphthol. The percentage of the dose converted to this metabolite is smaller the larger the dose of 2-naphthylamine given. On the other hand, when 2-acetamidonaphthalene is administered to the dog the proportion of conjugates of 2-amino-1-naphthol falls to less than 5 %.

Similar results have been obtained with the cat; the amount of 2-amino-1-naphthol produced from the free amine has been shown to be greatly in excess of that produced from the acetamido-derivative.

The amount of diazotizable amine in the hydrolysed urine of the dog is small when 2-naphthylamine is administered, but is relatively large when the 2-acetamido compound is fed.

These results were discussed, and further evidence was presented to show that other acetamido compounds are not de-acetylated by the dog as the primary stage in their metabolism.

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Uptake of $^{32}PO_4$ by Flavin-adenine Dinucleotide of Rat Liver *in vitro*. By W. GOOD and G. LEAF. (*Biochemistry Department, University of Glasgow*)

Hummel & Lindberg (1949) reported that when rabbit liver mince was incubated with $^{32}PO_4$, flavin-adenine dinucleotide (FAD) took up ^{32}P with almost the same rapidity as adenosinetriphosphate (ATP). This has been confirmed using rat liver. In contrast to FAD and ATP, coenzyme I, adenylic acid and flavin mononucleotide do not appear to exchange their phosphorus under the same conditions. The implications of these findings were discussed.

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The Synthesis and Secretion of Amylase by Pigeon Pancreas Slices *in vitro*. By L. E. HOKIN.* (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Sheffield*)

The incorporation of a variety of labelled amino-acids into the proteins of tissue slices and homogenates (Melchior & Tarver, 1947; Anfinsen, Beloff, Hastings & Solomon, 1947; Winnick, Friedberg & Greenberg, 1947; Frantz, Zamecnik, Reese & Stephenson, 1948; Borsook, Deasy, Haagen-Smit, Keighley & Lowy, 1949) has been regarded as evidence of protein synthesis *in vitro*. However, a net increase of protein on incubation of an isolated tissue has not yet been reported. It was thought that a demonstrable net increase of a protein might be found upon incubation of a glandular tissue, capable of rapidly synthesizing enzymes which can be quantitatively determined.

Pigeon pancreas slices, which were depleted of their amylase content by supplying the birds with abundant food and injecting intramuscularly 0·1–0·15 mg. of carbamylcholine about 1 hr. before killing, were incubated at 40° under various

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conditions. The amylase activities of the medium and tissue were measured by the method of Smith & Roe (1949). By this procedure synthesis as well as secretion of amylase could be studied.

A progressive increase in 'total amylase activity' (sum of activities of tissue and medium) was observed when slices were incubated aerobically in saline media containing glucose. A greater increase occurred in serum containing added glucose. The increase was greatest (over 100% in some experiments after 2 hr. incubation) when a mixture of amino-acids was added (see Table 1). There was no increase in total amylase activity under anaerobic conditions, or in the presence of 2:4-dinitrophenol (10^{-4}M), cyanide (10^{-4}M) or iodoacetate (10^{-4}M). These observations indicate that the formation of amylase depended on a supply of energy and suggest that the increase in amylase activity represented a true synthesis of enzyme and not an activation of a closely related precursor.

In the presence of cholinergic drugs the fraction of amylase in the medium was significantly increased. This increase did not occur anaerobically or in the presence of 2:4-dinitrophenol (10^{-4}M) or cyanide (10^{-4}M). Thus pigeon pancreas slices appear to be capable of actively secreting amylase *in vitro*. Under the conditions of these experiments synthesis of amylase was not appreciably affected by secretion. *In vitro* secretion of an enzyme (pepsin) has also been reported by Edwards & Edwards (1949).

Table 1. *Synthesis and secretion of amylase by pigeon pancreas slices* in vitro

(2 hr. aerobic incubation. 0·2% glucose.)

Exp. no.	Conditions	Amylase activity (units of Smith & Roe mg. initial dry wt.) Medium	Tissue	Total
1	Unincubated slice	—	59	59
	Incubated in bicarbonate saline	27	40	67
	Incubated in inactivated sheep serum	26	67	93
	Incubated in bicarbonate saline with 0·4% tryptophan-supplemented acid casein hydrolysate	31	75	106
2	Unincubated slice	—	64	64
	Incubated in bicarbonate saline	28	78	106
	Incubated in bicarbonate saline with mixture of 21 amino-acids	32	115	147
	Incubated in bicarbonate saline with 0·4% tryptophan-supplemented acid casein hydrolysate	30	113	143

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Choline and Methionine in the Embryonated Hen Egg. By G. S. Boyd. (*Department of Biochemistry, University of Edinburgh*)

Seventy eggs incubated for periods between 0 and 21 days have been dissected after freezing in acetone-CO_2. Homogenized embryos and 'rests' ('rest' = yolk + white + allantoic and amniotic fluids + membranes) have been separately analysed for choline (method modified from Ramsay & Stewart, 1941, and Beattie, 1936) and methionine (McCarthy & Sullivan, 1941). In each case the substance is present practically entirely in chemical combination, choline as phospholipin, methionine in proteins.

In twenty unincubated eggs the total choline has been found to average 183 mg./egg (S.D. ± 22 mg.), and in eight unincubated eggs the total methionine has averaged 165 mg./egg (S.D. ± 11 mg.). Table 1 gives the results of the embryo analyses. Both compounds increase with incubation in the expected more or less smooth exponential manner, and methionine accumulates more rapidly than choline.

The analyses of the 'rests' show a progressive decrease, which in the case of methionine corresponds broadly to the increase in the embryo. In fact, up to the 18th day, the sum of the embryo and 'rest' methionine approximates closely to the total

methionine of unincubated eggs. At the end of incubation there is a fall, however, of about 25% in the total methionine of the egg, although the exponential increase in the embryo remains uninterrupted.

Table 1. *Analyses of embryos*

Incubation period (days)	Choline (mg./ embryo)	Methionine (mg./ embryo)
6	1	1
10	2	2·5
12	3	8
14	8	20
16	16	36
18	30	45
20	40	75

On the other hand, the total choline of the embryo and 'rest' is less than the choline of unincubated eggs as early as the 13th day, and by the 21st day the egg contains not more than 50% of the original 180 mg. choline. This decrease may be partly a coincidental reflection of the parallel change in lipoid phosphorus (Plimmer & Scott, 1908; Masai & Fukutomi, 1923; confirmed in this laboratory), but it appears probable also that choline is the only donor of methyl groups in the egg at least until the last 2 days of incubation. Such quantitatively important compounds as creatine and creatinine do not, however, account for more than 25% of the choline which disappears, even when the assumption is made that only one of the three choline methyl groups is available for transmethylation (cf. du Vigneaud, Chandler, Simmonds, Moyer & Cohn, 1946).

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The Behaviour of β-Glucuronidase and Nucleic Acids in Rat Liver during Growth. By G. T. Mills, Evelyn E. B. Smith, Beatrice Stary and I. Leslie. (*Biochemistry Department, University of Glasgow*)

A study has been made of the β-glucuronidase concentration in the livers of growing rats from before birth to maturity and in the livers of rats following subtotal hepatectomy.

It has been found that the liver β-glucuronidase concentrations are low in foetal rats, and that after birth there is a rise to a maximum around 20–40 days followed by a slight decline to an adult level above that for animals at birth. An examination of our data by the allometric method, which was first employed by Huxley (1924), indicates that the accumulation rate of β-glucuronidase is greater than that for liver tissue during the growth of the animal. However, the presence of other variables obscures the picture when liver weight is used as a base line and a much more precise index of reference than this is required.

Davidson & Leslie (1950) have demonstrated the value of the constancy of the deoxyribonucleic acid (DNA) content of the cell nucleus for a single species as an index of reference when studying growth phenomena. In the present work, by using the DNA content of the tissues as an indicator of cell number, along with the allometric method of analysis, the results indicate that the β-glucuronidase content per cell increases up to about 18 days postpartum and thereafter remains constant, and it is after this time that the growth rate of the liver is maximum. Our data reveal no relation between the rate of cell proliferation and the β-glucuronidase content per cell in the rat liver.

A study of rat liver regenerating after subtotal hepatectomy leads us to the same conclusion. In these experiments when the results are calculated on the basis of the DNA content of the tissue, a constant β-glucuronidase content per cell is found throughout the whole period of regeneration. During the 3 days following the operation when regeneration is most intense, the protein concentration of the liver is increasing rapidly while the β-glucuronidase concentration remains constant. It is only at about 6 days, when the protein concentration has returned to a constant level and regeneration is about 80% complete, that there is any significant rise in β-glucuronidase concentration.

The situation in the rat would therefore appear to be different from that in the mouse, where Levvy, Kerr & Campbell (1948) and Kerr, Campbell & Levvy (1949, 1950) have recorded a connexion between cell proliferation and the glucuronidase concentration of an organ. Further work is in progress to determine whether or not this is a true species difference.

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The β-Glucuronidase of *Escherichia coli*. By EVELYN E. B. SMITH and G. T. MILLS. (*Biochemistry Department, University of Glasgow*)

It was recently shown by Buehler, Katzman & Doisy (1949) that certain strains of *Escherichia coli* will produce β-glucuronidase, and that this production is stimulated by the presence of menthyl glucuronide in the medium.

With the strains of *Esch. coli* which have been examined in this laboratory, the presence of menthyl glucuronide would appear to be essential for the production of glucuronidase. On agar or in peptone medium, no glucuronidase is produced, while transference from the peptone to a similar medium containing 0·1 % menthyl glucuronide stimulates glucuronidase production within 24 hr. For maximal production, growth in a medium containing 0·5 % menthyl glucuronide is essential. Retransference of the adapted organism to an agar slope results in a 'lag phase' requiring several subcultures in 0·1 % menthyl glucuronide to restimulate enzyme production. Growth is maintained in the adaptive state in a medium containing 0·1 % menthyl glucuronide, but in media containing 0·5 % menthyl glucuronide, the concentration of free menthol liberated enzymically becomes sufficient to inhibit growth completely.

It has been found that approximately 50 % of the glucuronic acid present may be utilized by the organism during a period of 2 days' growth. Menthyl glucuronide, however, cannot be utilized as the sole source of carbon.

The rate of production of glucuronidase varies depending on the strain of *Esch. coli* used. In the strain investigated, enzyme production was maximal within 2–3 days (0·5 % menthyl glucuronide), while in that kindly provided by Dr H. J. Buehler, St Louis School of Medicine, U.S.A., at least 10 days' growth in a similar medium was required to produce an equivalent enzyme preparation. Adaptation in the latter instance was also more prolonged.

In attempts to render the preparation cell-free by filtration, it was found that passage through a Seitz filter resulted in a complete loss of activity. The enzyme preparation was rendered cell free by centrifugation. Ultrasonic disintegration of the bacterial cell results in no increase in glucuronidase activity.

Dialysis resulted in a 95 % loss of activity, which was not regained by combining the enzyme and dialysate, nor did the addition of glycine (0·01 M) reactivate the enzyme. In this respect the glucuronidase under study differs from that obtained by Buehler *et al.* (1949), which was apparently unaffected by dialysis.

The pH optimum for the hydrolysis of phenolphthalein glucuronide (0·00025 M) by this enzyme in phosphate-citrate buffer is 6·2, and the isoelectric point determined by the suramin method of Wills & Wormall (1950) is 5·6. Saccharate at a concentration of 10^{-3} M shows marked inhibition.

Further data were presented on the purification and properties of this enzyme.

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2:4-Dinitrophenol in the Study of Pathways of Pyruvate Metabolism in Lactating Mammary Tissue. By C. TERNER. (*National Institute for Research in Dairying, University of Reading*)

In order to deplete lactating mammary tissue of preformed substrates, slices were shaken in three changes of ice-cold saline (Krebs & Eggleston, 1940) and then incubated at 37° for 1–2 hr. in fresh saline in equilibrium with 100 % O_2. After a further washing in cold saline, 100–150 mg. portions of blotted slices were incubated in the Warburg apparatus. Depleted rat mammary tissue had a low endogenous Q_{O_2} (−2 to −3) compared with untreated tissue (Q_{O_2}, −4 to −6). The small increase of Q_{O_2} of depleted tissue on addition of glucose or pyruvate was prevented by 0·0025 to 0·01 M-malonate. When fumarate (0·002 M) was present, addition of pyruvate raised the Q_{O_2} to the same level as in untreated

portions of the same tissue (Q_{O_2}, −6 to −11; cf. Folley & French, 1949), and malonate did not prevent the increase of Q_{O_2} (cf. Krebs & Eggleston, 1940). Depleted slices and also homogenates oxidized succinic acid.

Addition of pyruvate in the presence of fumarate to depleted sheep mammary tissue usually increased the Q_{O_2}, but sometimes the increase was small or absent. However, subsequent addition of 2×10^{-4}M-2:4-dinitrophenol (DNP) increased the Q_{O_2} by 50–100%. A similar increase in Q_{O_2} in the presence of pyruvate and DNP was observed with rat, rabbit and goat mammary slices; there was, however, no corresponding increase in the rate of utilization of pyruvate, the ratio $\Delta Q_{O_2} : Q_{pyruvate}$ being less than 1 in the absence, and approaching 2 in the presence of DNP, indicating more complete oxidation of pyruvate in the presence of DNP. Representative results are shown in Table 1. Inhibitors of reactions of the Krebs cycle, malonate (0·002–0·01M), and fluoroacetate (0·005–0·01M) reduced both oxygen uptake and pyruvate utilization (see Bartlett & Barron, 1947; Liébecq & Peters, 1949).

Since DNP has been shown to inhibit synthetic reactions (Clifton, 1946) and to prevent phosphorylation (Loomis & Lipmann, 1948), these findings suggest that, apart from being oxidized via the Krebs cycle, pyruvate can be utilized in synthetic reactions in the mammary gland. In isotope experiments, pyruvate has been shown to be a source of fatty acid carbon *in vivo* (Anker, 1948) and in rat liver slices *in vitro* (Brady & Gurin, 1950). Fat synthesis by mammary tissue *in vitro* is indicated by the work of Folley & French (1950) in which utilization of acetate or glucose with high R.Q. is demonstrated. It is, therefore, of interest that an increase of Q_{O_2} on addition of DNP could also be observed in rabbit mammary tissue incubated with glucose plus acetate (Table 1), but not with glucose alone. Preliminary R.Q. measurements by the direct method of Warburg indicated a high R.Q. in the presence of pyruvate in sheep and rat mammary tissue which was reduced on addition of DNP. Acetoacetate did not accumulate in the presence or absence of DNP, and the accumulation of acetate is unlikely in view of its ready utilization (Folley & French, 1950).

The possibility of pyruvate being used for the synthesis of fatty acids by mammary tissue is being further investigated.

Table 1. *Effect of* 2:4-*dinitrophenol on metabolism of lactating mammary tissue*

(Rabbit mammary gland slices, incubated at 37° in phosphate saline without Ca (Krebs & Eggleston, 1940); gas, 100% O_2; Fumarate (0·002M) in all cups. Additions: pyruvate (0·005M), DNP (2×10^{-4}M), glucose and acetate (0·01M).)

Cup no.	Substrates added	Q_{O_2} during period (min.) 10–40	40–70	70–100	$Q_{pyruvate}$	$\frac{\Delta Q_{O_2}}{Q_{pyruvate}}$
1	None	−3·0	− 2·9	− 2·9	—	—
2	Pyruvate at 40 min.	−3·2	− 8·4	− 8·4	−6·1	0·85
3	Pyruvate at 40 min.	−2·9	− 6·4	− 6·4	−3·8	0·92
4	Pyruvate, DNP at 40 min.	−3·0	−15·1	−15·1	−7·8	1·55
5	Pyruvate, DNP at 40 min.	−3·2	−15·8	−15·8	−7·9	1·60
6	Glucose + acetate at 40 min. DNP at 70 min.	−3·1	−10·2	−16·9	—	—
7	Glucose + acetate at 40 min. DNP at 70 min.	−3·1	−12·0	−17·7	—	—

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The Metabolism of Acetate in Pigeon Breast Muscle. By MABEL DAVISON and Q. H. GIBSON. (*Department of Physiology, University of Sheffield*)

Acetate is oxidized fairly readily by intact muscle cells (Lorber, Lifson, Wood & Barcroft, 1945; Medes, Floyd & Weinhouse, 1946; Villee & Hastings, 1949). The experiments reported here show that small amounts of steam-volatile acid disappear from muscle mince suspensions incubated with added acetate. The addition of boiled muscle or liver extract increases the steam-volatile acid disap-

pearance. This disappearance, if due to complete oxidation of acetate, would account for 6–15% of the total O_2 uptake. When either $^{14}CH_3COONa$ or $CH_3{}^{14}COONa$ was used, $^{14}CO_2$ was present in the respiratory CO_2. The effect of boiled tissue extract was to increase $^{14}CO_2$ formation as well as steam-volatile acid disappearance (Table 1). Succinate was isolated from experiments carried out with $^{14}CH_3COONa$ in the presence of 0·01 M-malonate. The succinate was found to be radioactive and to have a higher specific activity than that of the respiratory CO_2 (Table 2). This is regarded as evidence of formation of succinate from acetate in pigeon breast-muscle suspension. Boiled tissue extract increases the amount of labelled succinate formed.

Table 1. *Disappearance of steam-volatile acid and oxidation of acetate by minced pigeon breast-muscle suspensions*

(400 mg. wet weight muscle in 3 ml. phosphate saline, incubated 2 hr., 39°. Boiled tissue extracts (BTE) prepared from pigeon breast muscle, pigeon and rabbit liver. CH_3COONa oxidized calculated from $^{14}CO_2$ activity of respiratory CO_2.)

	Acetate oxidized (μmol.)	No. obs.	Steam-volatile acid disappearing (μmol.)	No. obs.
$CH_3{}^{14}COONa$				
No BTE	1·5±0·2	5	1·0±0·3	5
With BTE	3·3±0·4	10	3·8±0·5	8
$^{14}CH_3COONa$				
No BTE	1·0±0·2	7	1·1±0·2	7
With BTE	2·5±0·2	6	4·1±0·6	6

Table 2. *Formation of succinate from acetate by pigeon breast muscle*

(0·01 M-malonate, 30 μmol. fumarate/cup. Other conditions as Table 1. Acetate added gave 8900 counts/min. as $BaCO_3$ at infinite thickness. All counts expressed as counts/min. at infinite thickness, corrected for background, 11 counts/min.).

	Respiratory CO_2		Succinate	
	Counts/min.	Acetate (μmol.)	Counts/min.	Acetate (μmol.)
No BTE	20	0·1	114	0·6
BTE	29	0·1	251	1·3

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Observations on the Polysaccharides Composing the Cell Wall of Baker's Yeast. By D. J. Bell and D. H. Northcote. (*School of Biochemistry, University of Cambridge*)

The 'erythrocellulose' of Salkowski (1894) has been shown by Zechmeister & Toth (1934) and by Hassid, Joslyn & McCready (1941) to consist of two initially insoluble polysaccharides apparently both based on D-glucose. One of these is resistant to mild acid hydrolysis, the other is not. The latter has been termed 'pseudoglycogen' by McAnally & Maclean (1937). The Hungarian and American workers have both shown that the resistant 'glucan' is largely based on the 1:3-β-linked D-glucose radical. Bell & Northcote (1950), using chromatographic methods, have succeeded in accounting for nearly 100% of this substance and have shown that the polysaccharide is highly branched as follows: the average chain length is 9–10 radicals, the inter-radical links are 1:3, and the inter-chain link is 1:2. We have further examined the unhydrolysed pseudoglycogen which seems to be highly branched (mean chain length approx. 11 D-glucose radicals), it loses its iodine staining power under the action of salivary amylase and superficially resembles animal glycogens.

Since morphological relationships are assumed between red algae, yeasts and the algae components of lichens, this occurrence of the β-1:3 D-glucose linkage in yeast glucan is of interest. In red algae, 'Floridean starch' (Barry, Halsall, Hirst & Jones, 1949) and in lichenin from Iceland moss (Boissonas, 1947) these links are also found. In brown algae the polysaccharide laminarin possesses this link probably exclusively. The 1:2-β-D-glucan link has also been found in the 'hemicellulose' fraction from Iceland moss (Granichstädten & Percival, 1943). It should be noted that cell walls of brown algae appear to be composed of cellulosic material similar to terrestrial cellulose (Percival & Ross, 1949). A presumed reserve polysaccharide of brown algae, laminarin, is entirely composed of glucose radicals 1:3 linked according to Barry (1943). No evidence of the presence of amino sugars, alleged to occur in cell walls of fungi, has been obtained among yeasts or algae.

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