activators though less so than magnesium. Potassium ions are inactive in this respect.

3. The pH optimum of the enzyme for the forward reaction, i.e. the splitting of phosphocreatine in the presence of adenylic acid lies in the range of 5·9–7; phosphocreatine is split optimally at the same pH range when adenosinediphosphate is used as the phosphate acceptor.

4. Preparations from brain also catalyse the synthesis of phosphocreatine from creatine and adenosinetriphosphate above pH 8·2.

5. None of the drugs of central action which were examined had any significant effect on the activity of the enzyme. Iodoacetate inhibited it completely above a concentration of 0·5 mM and fluoride to a much less marked degree.

I wish to thank Dr McIlwain for having suggested the problem and for many helpful discussions.

REFERENCES

Banga, I. (1943). *Stud. Inst. med. Chem. Univ. Szeged, Hung.*, **3**, 59.

Bielschowsky, M. (1950). *Biochem. J.* **47**, 105.

Dawson, R. M. C. & Richter, D. (1950). *Amer. J. Physiol.* **160**, 203.

Ennor, A. H. & Stocken, L. A. (1948). *Biochem. J.* **43**, 190.

Gore, M. B. R. (1951). *Biochem. J.* **50**, 18.

Hawk, P. B., Oser, B. L. & Summerson, W. H. (1947). *Practical Physiological Chemistry*, 12th ed. London: Churchill.

Klein, J. R. & Olsen, N. S. (1947). *J. biol. Chem.* **167**, 747.

Krebs, H. A. (1931). *Biochem. Z.* **234**, 278.

Lehmann, H. (1935). *Biochem. Z.* **281**, 271.

Lehmann, H. (1936). *Biochem. Z.* **286**, 336.

Lohmann, K. (1934). *Biochem. Z.* **271**, 264.

McIlwain, H. (1950). *Brit. med. Bull.* **6**, 301.

McIlwain, H., Anguiano, G. & Cheshire, J. D. (1951). *Biochem. J.* **50**, 12.

McIlwain, H. & Gore, M. B. R. (1951). *Biochem. J.* **50**, 24.

Meyerhof, O. & Wilson, J. R. (1948). *Arch. Biochem.* **19**, 502.

Stone, W. E. (1943). *J. biol. Chem.* **149**, 29.

Stone, W. E., Webster, J. E. & Gurdjian, E. S. (1945). *J. Neurophysiol.* **8**, 233.

Effects of Insulin and of Glycerol *in vitro* on the Incorporation of [*carboxy*-^{14}C]acetate into the Fatty Acids of Lactating Mammary Gland Slices with Special Reference to Species Differences

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Udder slices from lactating ruminants (cow, ewe, goat) have been shown actively to utilize acetate as sole substrate with R.Q. > 1 (Folley & French, 1950), from which it was concluded that udder tissue of the ruminant can synthesize fat from acetate alone. On the other hand, slices of lactating mammary gland from non-ruminants (rat, rabbit) were inert to acetate, which, however, was utilized in the presence of glucose, the R.Q. then being of the same order (> 1) as in glucose alone. Moreover, addition of glucose to the medium increased the amount of acetate used by mammary gland slices from ruminants (sheep) and the R.Q. was increased. This was taken to indicate that glucose increases the utilization of acetate for fat synthesis under these conditions.

The effect of insulin *in vitro* on fat synthesis by mammary gland slices has also been studied by respiratory measurements. Addition of insulin to the incubation medium resulted in a marked increase in the R.Q., $-Q_{O_2}$ and acetate uptake of rat mammary gland slices metabolizing mixtures of acetate and glucose, but had no effect on the respiration of the slices in acetate alone, from which it was concluded that in rat mammary tissue insulin enhances fat synthesis from acetate in the presence of glucose, but evokes no fatty acid synthesis in acetate alone (Balmain, French & Folley, 1950; Balmain & Folley, 1951). Sheep udder slices, on the other hand, were inert to insulin both in acetate alone and in acetate plus glucose (Balmain & Folley, 1951). The increase in the R.Q. of mammary gland slices from lactating rats in the presence of insulin has been confirmed by Hills & Stadie (1952).

Glycerol has been found to influence the metabolism of acetate plus glucose by rat mammary gland slices in a way similar to insulin in all respects save that the increase in R.Q. was less marked and, more important, glycerol did not increase $-Q_{\text{glucose}}$ while insulin did. This finding was interpreted as

showing that glycerol increased the rate of fatty acid formation by the slices and thus fat synthesis in the mammary gland might be limited by the amount of glycerol available (Balmain & Folley, 1951).

Since the above conclusions rested largely on R.Q. determinations which are not universally accepted as yielding unequivocal information on the type of reaction occurring within an organ or tissue, it was considered advisable also to investigate these questions by tissue-slice experiments using isotopically labelled acetate. The first of such experiments, in which mammary gland slices from lactating rats were incubated with glucose and [*carboxy*-^{14}C]acetate, the water of the medium being labelled with tritium, showed that the fatty acids synthesized by the slices *in vitro* contained appreciable amounts of tritium and ^{14}C and that the content of both isotopes was increased in presence of insulin (Balmain, Folley & Glascock, 1951). The results of the present, more extensive, experiments, which have been reported in preliminary form elsewhere (Balmain, Folley & Glascock, 1952), confirm the above-mentioned deductions made from respiratory and acid-change measurements in every particular.

METHODS

Experimental animals: incubation of slices. The animals used were primiparous Norway rats (seven to eight young each) killed by dislocation of the neck after 13–15 days of lactation, and three primiparous lactating ewes (one lamb each) shot through the brain after 23–27 days of lactation. In the case of the ewes, each udder, immediately after removal, was dissected into its two constituent glands and separate experiments set up with tissue from each half, making, as in the case of the rats, six experiments in all.

Mammary tissue was sliced with a Stadie & Riggs (1944) microtome. Pooled abdominal mammary tissue from two animals was used for each experiment with rats, batches of slices weighing 2 g. being shaken for 3 hr. at 37° in 100 ml. Warburg flasks containing 20 ml. Krebs bicarbonate saline, the gas phase being 95% O_2 and 5% CO_2 (v/v). The medium contained 0·02 M-sodium acetate or 0·02 M-sodium acetate + 0·3% glucose. The acetate was labelled with $CH_3{}^{14}COONa$, the total radioactivity in each 20 ml. medium being 1·0 μc. In each experiment four flasks were set up containing acetate alone and four containing acetate + glucose. In one flask of each of these two series of four the tissue was killed at the outset by the addition of HCl to a concn. of 0·3 N. Crystalline insulin (Novo), free from glycogenolytic factor, was added to the medium in one of the remaining three flasks to a final concentration of 0·05 mg. (approx. 1 i.u.)/ml. and glycerol (final concn. 0·2%) to the medium in another. No addition was made to the fourth flask. The flasks were attached to Warburg manometers so that the steady rise in pressure, indicative of the high R.Q. exhibited by rat mammary gland slices under these conditions, and the enhanced rate of pressure rise due to the addition of insulin or glycerol, could serve as convenient indicators of the progress of the experiment.

The experiments on sheep tissue (3 g. batches of slices) were set up similarly except that no manometric observations were made; the incubations (3 hr.) were carried out in ordinary conical flasks each with a rubber bung carrying a glass tube, dipping under the surface of the medium, through which the gas mixture was bubbled (with constant shaking) throughout the incubation period.

Isolation of total fatty acids. The method used for the isolation of the total fatty acids of the slices was modified from that of Popják & Beeckmans (1950). At the end of the incubation period the tissue was killed by addition of HCl to a concn. of 0·3 N and the contents of each flask homogenized in a Folley & Watson (1948) homogenizer. After neutralization to litmus the mixture was taken practically to dryness under reduced pressure at 40° and the residue refluxed five times for 5 min. with successive 25 ml. lots of ethanol-ether, 3:1 (v/v). The decanted extracts were combined and taken to dryness under reduced pressure at 40° and traces of water removed by distilling 50 ml. benzene from the residue. The residue was refluxed five times for 5 min. with 25 ml. portions of light petroleum (b.p. 40–60°). The combined, filtered extracts were taken to dryness (reduced pressure), and the residue refluxed for 24 hr. with 5 ml. ethanolic KOH (1 vol. 40% KOH:10 vol. absolute ethanol). The solution of soaps was diluted with 10 ml. 50% (v/v) ethanol and extracted with 5 × 50 ml. light petroleum. The solution was then acidified with H_2SO_4 and extracted with 3 × 25 ml. light petroleum. The extract was washed with water until the washings were neutral, 1 drop of phenolphthalein added, and the liquid titrated to a faint permanent pink with $Ca(OH)_2$ solution. The light petroleum was distilled from the solution of Ca salts of the fatty acids and the latter were evaporated to dryness on the steam bath. The Ca salts were not washed with unlabelled acetate since the 'blank' values (Table 1) gave no reason to suspect that they were contaminated with $CH_3{}^{14}COONa$. The samples so obtained were counted with an end-window counter at infinite thickness on 1·0 cm.^{2} disks and the results expressed as counts/min./mg. C by use of an appropriate factor.

Isolation of glycerol. Glycerol was isolated as the tri-*p*-nitrobenzoate from the pooled saponification mixtures after extraction of the fatty acids, there being eight samples (blank, control, insulin, and glycerol each with acetate and glucose + acetate, respectively, as substrates) for each species (rat and sheep). Each solution was made just alkaline to phenolphthalein by addition of KOH and evaporated to dryness on the steam bath. The solid residue was extracted by refluxing with five successive portions of ethyl acetate (25 ml. each) and the solvent distilled off under reduced pressure. The residue was dissolved in 5 ml. pyridine, slightly more than the theoretical amount of *p*-nitrobenzoyl chloride added, the mixture refluxed for 5 min. and cooled for 1 hr. in the refrigerator. Water was added and the glyceryl tri-*p*-nitrobenzoate collected on a sintered-glass filter. The precipitate was washed on the filter with 0·1 N-NaOH and then thoroughly washed with water. It was then washed with 5 ml. 95% ethanol added dropwise and finally given ten washes with 2 ml. portions of acetone, the precipitate being thoroughly stirred each time. The final product dried over P_2O_5 *in vacuo* had m.p. 188° and was assayed for ^{14}C.

RESULTS

Effect of glucose on incorporation of acetate carbon into fatty acids. A comparison of the incorporation of acetate carbon into the total fatty acids of the

mammary gland slices in the presence of acetate and of acetate plus glucose, respectively, is given for both species in Table 1. The results show in the first place that rat mammary gland slices in acetate alone incorporate no acetate carbon into fatty acids under our conditions, while sheep mammary gland slices incorporate appreciable amounts. In the presence of glucose, however, rat tissue is able to utilize considerable amounts of acetate carbon for fatty acid synthesis and, moreover, the incorporation effected by sheep udder slices is increased 2–3 times. The stimulating effect of glucose on fatty acid synthesis from acetate by mammary slices is thus very marked in both rat and sheep mammary tissue.

Effect of insulin on incorporation of acetate carbon into fatty acids. The effect of insulin on the incorporation of acetate carbon into the fatty acids of rat mammary slices is shown in Table 2, and of sheep udder slices in Table 3. It will be seen that insulin evoked no fatty acid synthesis from acetate in rat mammary tissue incubated with acetate alone but, in the presence of glucose, insulin markedly increased the acetate incorporation in all experiments. In sheep udder tissue, irrespective of whether glucose was present, insulin had no detectable effect on fatty acid synthesis from acetate.

Table 1. *Effect of glucose on the incorporation of* ^{14}C *into fatty acids of mammary gland slices*

(The results in this and the following tables are expressed as counts/min./mg. fatty acid C.)

	Rat mammary tissue Exp. no.						
Substrate	1	2	3	4	5	6	Blank values*
[*carboxy*-^{14}C]acetate	50	43	40	50	58	40	11–58
[*carboxy*-^{14}C]acetate + glucose	5951	3805	5886	3532	3215	1069	0–40
	Sheep mammary tissue Exp. no.†						
	1L	1R	2L	2R	3L	3R	
[*carboxy*-^{14}C]acetate	3334	3539	2660	2948	4144	4018	29–76
[*carboxy*-^{14}C]acetate + glucose	9533	9911	8975	10249	8086	7441	14–54

* The blank values also apply to the results in the following tables.
† 1L refers to the left udder-half of sheep no. 1, etc.

Table 2. *Effect of insulin on the incorporation of* ^{14}C *into fatty acids of rat mammary gland slices*

	Exp. no.					
Addition	1	2	3	4	5	6
	Substrate: [*carboxy*-^{14}C]acetate					
Insulin	86	40	97	43	58	58
None	50	43	40	50	58	40
	Substrate: [*carboxy*-^{14}C]acetate + glucose					
Insulin	9061	7567	10847	6383	7132	1991
None	5951	3805	5886	3532	3215	1069

Table 3. *Effect of insulin on the incorporation of* ^{14}C *into fatty acids of sheep udder slices*

	Exp. no.*					
Addition	1L	1R	2L	2R	3L	3R
	Substrate: [*carboxy*-^{14}C]acetate					
Insulin	4363	3330	2167	2671	3442	3658
None	3334	3539	2660	2948	4144	4018
	Substrate: [*carboxy*-^{14}C]acetate + glucose					
Insulin	9176	10195	9115	7729	7722	7913
None	9533	9911	8975	10249	8068	7441

* 1L refers to the left udder-half of sheep no. 1, etc.

Effect of glycerol on the incorporation of acetate into fatty acids. Tables 4 and 5 show the effects of glycerol on the utilization of acetate carbon for fatty acid synthesis by rat and sheep mammary tissue respectively. In the case of rat tissue in acetate alone, the results indicate that addition of glycerol evoked a slight utilization of acetate carbon for fatty acid synthesis. In glucose plus acetate, however, glycerol almost doubled the utilization of acetate for fatty acid synthesis, the effect being

quite as large as that of insulin. In this connexion it may be noted that in respiratory experiments (Balmain & Folley, 1951) glycerol has given somewhat smaller increases in R.Q. than insulin. In the case of sheep udder tissue in acetate alone, glycerol was effective in markedly increasing the amount of ^{14}C found in the fatty acids, but was ineffective in the presence of glucose.

R.Q. whilst an increase only in turnover rate would not do so; hence the present results taken in conjunction with our previous respiratory measurements leave little doubt that in the present experiments substantial incorporation of ^{14}C into fatty acids is indicative of an increase in the rate of net fatty acid synthesis. The two sets of results, obtained by quite independent techniques, thus rein-

Table 4. *Effect of glycerol on the incorporation of* ^{14}C *into fatty acids of rat mammary gland slices*

	Exp. no.					
Addition	1	2	3	4	5	6
	Substrate: [*carboxy*-^{14}C]acetate					
Glycerol	209	94	151	86	115	158
None	50	43	40	50	58	40
	Substrate: [*carboxy*-^{14}C]acetate + glucose					
Glycerol	10282	8050	10566	6804	7657	2088
None	5951	3805	5886	3532	3215	1069

Table 5. *Effect of glycerol on the incorporation of* ^{14}C *into fatty acids of sheep udder slices*

	Exp. no.*					
Addition	1L	1R	2L	2R	3L	3R
	Substrate: [*carboxy*-^{14}C]acetate					
Glycerol	6880	5040	6534	5731	5753	5148
None	3334	3539	2660	2948	4144	4018
	Substrate: [*carboxy*-^{14}C]acetate + glucose					
Glycerol	8878	9209	8752	8255	7477	7301
None	9533	9911	8975	10249	8068	7441

* 1L refers to the left udder-half of sheep no. 1, etc.

Origin of glyceride glycerol. All samples of glyceryl tri-*p*-nitrobenzoate were virtually non-radioactive, indicating that under our conditions mammary gland slices of either species are unable to incorporate acetate carbon into glycerol.

DISCUSSION

All the deductions, mentioned in the introductory section of this paper, regarding the ability of mammary gland slices to utilize acetate for fatty acid synthesis *in vitro*, and the effects or lack of effects of glucose, glycerol and insulin upon this process in mammary tissue of ruminants and non-ruminants, have been confirmed by the present results; that is, in so far as they show that acetate carbon is incorporated into the fatty acids of lactating mammary tissue under certain defined conditions and that the rate of incorporation is increased in some circumstances by addition of the above-mentioned agents. It is not possible to tell from these results whether in any instance there has been an increased net synthesis of fatty acids or merely an increased turnover rate. However, an increased net synthesis would probably increase the R.Q. whilst an increase only in turnover rate would not do so; hence the present results taken in conjunction with our previous respiratory measurements leave little doubt that in the present experiments substantial incorporation of ^{14}C into fatty acids is indicative of an increase in the rate of net fatty acid synthesis. The two sets of results, obtained by quite independent techniques, thus reinforce and complement one another, and it may be concluded that useful deductions about fat synthesis by mammary gland slices from lactating animals may be drawn from R.Q. measurements with fair confidence.

Certain features of the results are worthy of comment. Addition of glycerol causes a small but definite incorporation of acetate carbon into the fatty acids of rat mammary slices in presence of acetate alone, a substrate towards which the slices are totally inert in absence of glycerol. In view of the marked utilization of acetate for fatty acid synthesis by rat mammary tissue in presence of glucose it would seem reasonable to suppose that rat mammary slices can convert glycerol to glucose or to some metabolite of glucose through which glucose stimulates fatty acid synthesis from acetate. The results show, however, that this conversion must be very slow.

As regards the mechanism of the stimulation of fatty acid synthesis by glucose, it was suggested by Folley & French (1949*b*, 1950) that if, as it is reasonable to suppose, glycerol can be a limiting factor in the synthesis of glycerides by mammary tissue and

hence, at one remove, of fatty acids, the favourable effect of glucose might be due to its breakdown to glycerol. Indeed, French & Popják (1951) later demonstrated the active incorporation of carbon from [^{14}C]glucose into the glycerol of the milk glycerides in the living rabbit. However, since, as shown by the present work, the effect of glycerol on the utilization of acetate for fatty acid synthesis by rat mammary slices is very slight in comparison with that of glucose and, moreover, since both isotope and respiration studies demonstrate that in rat mammary tissue glycerol is effective when glucose is present, this cannot be the complete explanation of the glucose effect, at any rate as regards rat mammary tissue. On the other hand, this theory would suffice to explain our observations on sheep udder slices since in this tissue glycerol stimulates fat synthesis in acetate alone, but in acetate plus glucose causes no further enhancement beyond that due to glucose. Indeed, if anything, the results in all six experiments (Table 5) show that glycerol slightly inhibits the incorporation of ^{14}C into the fatty acids in presence of acetate plus glucose.

The absence of radioactivity from all the samples of glycerol isolated from the glycerides of the slices is of interest. It indicates that mammary tissue of ruminant and non-ruminant alike cannot *in vitro* incorporate acetate carbon into glycerol. This is in contrast with recent findings in the lactating animal *in vivo*. Incorporation of ^{14}C from [*carboxy*-^{14}C]-acetate into the glycerol of the milk glycerides has been observed in the lactating goat (Popják, Glascock & Folley, 1952) and rabbit (French & Popják, 1951). That this can occur in the mammary gland itself is probable since the perfused isolated bovine udder incorporates acetate carbon into lactose (Cowie *et al.* 1951). The origin of the glycerol presumably used for glyceride formation by sheep udder slices actively metabolizing acetate alone (with R.Q. > 1, as may be concluded from the results of Folley & French, 1950, and therefore probably effecting net fatty acid synthesis), is accordingly rather puzzling. It is of course possible that in these slices free fatty acids not esterified with glycerol are accumulating—and the fact that glycerol does appear to be a limiting factor in this particular system is in accord with this suggestion—but perhaps it is more likely that the necessary glycerol is formed from some precursor stored in the tissue cells.

The differences between mammary tissue from ruminants and non-ruminants in respect of their response to insulin *in vitro*, observed in our previous respiratory studies and now confirmed by isotope incorporation experiments, may be significant as regards the mechanism of the stimulating effect of insulin on fatty acid synthesis. Respiration studies indicate that mammary tissues from ruminants and non-ruminants may differ in their energy-yielding systems. Mammary tissue of the non-ruminant appears to obtain its energy from the assimilation of carbohydrate (Folley & French, 1949*a*); the ruminant udder, however, oxidizes acetate in preference to carbohydrate (Folley & French, 1950). Since fatty acid synthesis in the mammary gland is an energy-requiring process it is tempting to suggest that the insulin effect is exerted via the system which obtains energy from carbohydrate oxidation. This theory agrees with the fact that the insulin effect has been observed only with non-ruminant mammary tissue and then only in presence of glucose. It is true that Brady & Gurin (1950) found that insulin increases the incorporation of acetate carbon into the fatty acids of rat-liver slices metabolizing acetate as sole substrate, but the presence of carbohydrate stores in liver tissue from fed rats must not be overlooked in interpreting these findings. On the other hand, the similarity in the effects of glycerol and insulin on fat synthesis from acetate which emerges from the present experiments, even more clearly than from our earlier respiratory studies, suggests that, as pointed out previously (Balmain & Folley, 1951), the insulin effect in this system may possibly be mediated, partly at least, through the breakdown of glucose to glycerol which occurs in mammary tissue. Two observations on rat mammary slices are in accord with this theory: first that the effects of insulin and glycerol are not additive, and secondly that while insulin increases $-Q_{glucose}$ in acetate plus glucose, glycerol does not (Balmain & Folley, 1951). The fact that sheep udder slices in acetate plus glucose are inert not only to insulin but to glycerol as well would also fit in with this view. This very tentative hypothesis does not necessarily conflict with the findings of Chaikoff and his collaborators (Felts, Chaikoff & Osborn, 1951*a*, *b*; Osborn, Chaikoff & Felts, 1951) who showed that pretreatment of the donors with insulin diverted acetate (and the two-carbon fragment formed from pyruvate) from oxidation to fatty acid synthesis in liver slices from diabetic rats.

SUMMARY

1. The effects of insulin and of glycerol, added *in vitro*, on the incorporation of ^{14}C into the total fatty acids of slices of lactating mammary gland from the rat and sheep, incubated in [*carboxy*-^{14}C]acetate alone or with unlabelled glucose, have been studied.

2. Rat mammary gland slices incorporated no acetate carbon into the fatty acids in acetate alone, but incorporated considerable amounts in the presence of glucose. Sheep udder slices effected considerable fatty acid synthesis from acetate alone, but the utilization of acetate carbon for this purpose was greatly increased in presence of glucose.

3. Insulin did not alter the inactivity of rat mammary gland slices towards acetate as sole substrate, but in presence of glucose it considerably increased the ^{14}C found in the fatty acids. The incorporation of ^{14}C into the fatty acids of sheep udder slices was unaffected by insulin both in acetate alone and in acetate plus glucose.

4. Glycerol caused a slight incorporation of acetate carbon into the fatty acids of rat mammary gland slices in acetate alone, and in acetate plus glucose its stimulating effect on ^{14}C incorporation was as great as that of insulin. It stimulated the utilization of acetate for fatty acid synthesis by sheep udder slices in acetate alone but in acetate plus glucose its effect was slightly inhibitory.

5. Glyceride glycerol isolated as tri-*p*-nitrobenzoate from the neutral fat of the slices was not radioactive in any experiment. Mammary gland slices under our conditions do not incorporate acetate carbon into glycerol.

6. Deductions from respiratory and acid-change measurements as to the ability of mammary gland slices to utilize acetate for fatty acid synthesis *in vitro* and the effect of glucose, of insulin and of glycerol upon this process in ruminant and non-ruminant mammary tissue respectively have been confirmed. There is now little doubt that mammary gland slices are capable of effecting fatty acid synthesis from small molecules *in vitro*.

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REFERENCES

Balmain, J. H. & Folley, S. J. (1951). *Biochem. J.* **49**, 663.
Balmain, J. H., Folley, S. J. & Glascock, R. F. (1951). *Nature, Lond.*, **168**, 1083.
Balmain, J. H., Folley, S. J. & Glascock, R. F. (1952). *Biochem. J.* **50**, xxix.
Balmain, J. H., French, T. H. & Folley, S. J. (1950). *Nature, Lond.*, **165**, 807.
Brady, R. O. & Gurin, S. (1950). *J. biol. Chem.* **186**, 461.
Cowie, A. T., Duncombe, W. G., Folley, S. J., French, T. H., Glascock, R. F., Massart, L., Peeters, G. J. & Popják, G. (1951). *Biochem. J.* **49**, 610.
Felts, J. M., Chaikoff, I. L. & Osborn, M. J. (1951*a*). *J. biol. chem.* **191**, 683.
Felts, J. M., Chaikoff, I. L. & Osborn, M. J. (1951*b*). *J. biol. Chem.* **193**, 557.
Folley, S. J. & French, T. H. (1949*a*). *Biochem. J.* **45**, 117.
Folley, S. J. & French, T. H. (1949*b*). *Biochem. J.* **44**, xlv.
Folley, S. J. & French, T. H. (1950). *Biochem. J.* **46**, 465.
Folley, S. J. & Watson, S. C. (1948). *Biochem. J.* **42**, 204.
French, T. H. & Popják, G. (1951). *Biochem. J.* **49**, iii.
Hills, A. G. & Stadie, W. C. (1952). *J. biol. Chem.* **194**, 25.
Osborn, M. J., Chaikoff, I. L. & Felts, J. M. (1951). *J. biol. Chem.* **193**, 549.
Popják, G. & Beeckmans, M.-L. (1950). *Biochem. J.* **46**, 547.
Popják, G., Glascock, R. F. & Folley, S. J. (1952). *Biochem. J.* **52**, 472.
Stadie, W. C. & Riggs, B. C. (1944). *J. biol. Chem.* **154**, 687.

D-Amino-acid Oxidase in the Molluscan Liver

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The enzyme D-amino-acid oxidase was first found in mammalian liver and kidney (Krebs, 1933); it occurs in all vertebrates that have been examined. A review on D-amino-acid oxidases by Krebs (1948) refers to the occurrence of an enzyme of this type in bacteria and in certain strains of the mould *Neurospora*.

We have recently briefly reported that D-amino-acid oxidase is present in the liver of *Octopus vulgaris* and of *Sepia officinalis* (Blaschko & Hawkins, 1951). This appears to be the first time that the enzyme has been found in invertebrates. We now wish to report some observations on the occurrence of D-amino-acid oxidase in other classes of molluscs and also to describe some of the properties of this enzyme.

MATERIALS AND METHODS

Animals. Specimens of *Sepia officinalis* and *Octopus vulgaris* were dissected in Plymouth. The livers were immediately frozen in solid CO_2 and taken to Oxford in a thermos flask. They were then stored at $-10°$.

In the other species examined (*Mytilus, Anodonta, Helix*) the liver is not as well defined a structure as in the two species of cephalopods, and tissue used included parts of the alimentary canal. *Mytilus edulis* was also obtained from