Hormones and Liver Cytoplasm

3. SUCCINIC DEHYDROGENASE, NUCLEASES AND 'POLYMERIZED' RIBONUCLEIC ACID AS AFFECTED BY HYPOPHYSECTOMY, GROWTH-HORMONE TREATMENT AND ADRENALECTOMY

By BARBARA M. STEVENS* AND E. REID†

Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, S.W. 3

(Received 27 April 1956)

With changes in hormonal status, certain changes occur in the amount or distribution of adenosine triphosphatase, glucose 6-phosphatase and xanthine oxidase activities in rat-liver cytoplasm (Reid, O'Neal & Lewin, 1956). These studies have now been extended to include succinic dehydrogenase, deoxyribonuclease, and the two ribonucleases which appear to be present in liver-mitochondrial fractions (Lamirande, Allard, Costa & Cantero, 1954; Roth, 1954a).

Since adrenalectomy leads to a rise in the amount of ribonucleic acid (RNA) in the 'supernatant fraction' (Reid, 1955, 1956), and since the administration of cortisone leads to certain changes in the amount of 'polymerized' RNA in liver cytoplasm (Lowe, 1955), a study has also been made of hormonal effects on the amount of 'polymerized' RNA in the supernatant fraction.

EXPERIMENTAL

Animals and tissue fractionations

The general procedures were as described in the preceding paper (Reid, O'Neal & Lewin, 1956; see also Reid, 1956, and Reid, O'Neal, Stevens & Burnop, 1956), and may be summarized as follows. The experimental animals were young male rats which had been hypophysectomized or adrenalectomized 1½–3 weeks previously and had been maintained on a limited food ration; some of the hypophysectomized rats had received growth hormone (0.3 mg./day) for 8 days before autopsy. The animals were arranged in groups, each group including one or two control rats (intact or sham-operated). As in the preceding paper (Reid, O'Neal & Lewin, 1956), the results for the experimental rats were compared with those for the control rats studied simultaneously, the differences being averaged over the whole series. At least six untreated hypophysectomized rats, four hypophysectomized rats treated with growth hormone and six adrenalectomized rats were used in the study of each enzyme.

Liver homogenates, prepared in 0.25 M sucrose solution containing ethylenediaminetetraacetic acid (EDTA; 0.001 M), were subjected to differential centrifuging. After removal of a fraction containing the nuclei, three fractions (mitochondrial, microsomal and supernatant fractions) were

separated from the cytoplasmic material. The fractions were frozen and stored until required for enzymic assays; even with succinic dehydrogenase, the most labile of the enzymes studied, freezing and thawing did not impair the activity if carried out once only.

Enzymic assays and RNA determinations

Estimations of succinic dehydrogenase activity and of RNA content were usually carried out in duplicate, but triplicate estimations were performed in all nuclease assays, the procedures for which are necessarily somewhat empirical. With each enzyme studied, it was verified that activity was linearly related to time and to amount of tissue.

Succinic dehydrogenase. This enzyme was assayed by spectrophotometric measurement of the rate of reduction of cytochrome *c* (at 26°) in the presence of cyanide, essentially as described by Kuff & Schneider (1954) but with certain modifications (Duve, Pressman, Gianetto, Wattiaux & Appelmans, 1955). The final concentration of cytochrome *c* was increased to 0.5×10^{-4} M, and CaCl_2 was omitted (this omission being without effect on the level of activity). Immediately before each estimation, the diluted tissue sample was 'pre-incubated' for 30 min. at 0° in the presence of EDTA (0.001 M). Any 'endogenous' activity that was manifest in the absence of succinate was subtracted from the activity observed in the presence of succinate, these activities being estimated from graphical plots of the spectrophotometric readings.

Cytochrome *c* obtained from the Sigma Chemical Co. (St Louis, U.S.A.) was satisfactory for these assays. With cytochrome *c* preparations obtained from each of three other sources, little activity could be demonstrated, possibly because of rapid autoxidation (Henderson & Rawlinson, 1956).

Nucleases. Assays for deoxyribonuclease and acid ribonuclease followed the procedures of Duve *et al.* (1955), based on those of Schneider & Hogeboom (1952) but with an increased concentration of substrate and with suitable pre-treatment of the tissue preparation. This pre-treatment, the purpose of which was to liberate 'latent' activity, consisted in the present experiments of a freezing and thawing procedure (Gianetto & Duve, 1955), carried out eight times with the tissue preparation, which had first been diluted with water. After this pre-treatment, incubation was performed for 30 min. at 37° with shaking, the substrate being added either initially or, to the blanks, just before the final addition of 10% (w/v) perchloric acid. From the extinction at 260 mμ. of the acid-soluble material, activities were calculated as described by Duve *et al.* (1955).

* Royal Marsden Hospital Gordon Jacobs Fellow.

† British Empire Cancer Campaign Research Fellow.

The assays for alkaline ribonuclease were performed similarly, the incubation being performed in phosphate buffer at pH 8.2; but Mg^{2+} ions were omitted and the concentration of substrate was increased threefold (to a final concentration of 4 mg./ml. in the incubation mixture), in accordance with the procedure of Lamirande *et al.* (1954).

Herring-sperm deoxyribonucleic acid was used as the substrate for deoxyribonuclease. Difficulty was encountered in choosing a substrate for ribonuclease assays. Schneider & Hogeboom (1952) used RNA prepared from liver as the substrate for acid ribonuclease, but other authors have used yeast RNA in the assay of acid and alkaline ribonuclease activities (Roth, 1954*a*; Lamirande *et al.* 1954; Duve *et al.* 1955). In the present study, dialysed yeast RNA (Pharmaco-Chemical Products Co.) was used as the substrate for acid ribonuclease, but liver RNA (batch 65/1; prepared by Mr R. H. Pain) was used for alkaline ribonuclease. The activities of acid ribonuclease towards this batch of liver RNA and towards the yeast RNA were (in arbitrary units) 7 and 35 respectively, whereas the activities of alkaline ribonuclease (with the same tissue sample) were 29 and 2 respectively towards these two substrates. Different batches of liver RNA, prepared under essentially similar conditions, varied in their effectiveness as substrates, the activity of alkaline ribonuclease towards these samples being usually lower than that towards batch 65/1. Variations among liver RNA preparations have previously been encountered in studies with acid ribonuclease (private communication from Dr W. C. Schneider).

Total and 'polymerized' RNA. Total RNA was determined by measuring the extinction, at 260 $m\mu$., of acidified alkaline digests as in the experiments of Reid, O'Neal, Stevens & Burnop (1956). 'Polymerized' RNA, namely the RNA which is precipitable by ethanol from 10% (w/v) NaCl solution at 4° and which can be reprecipitated by trichloroacetic acid, was isolated as described by Lowe (1955), and was determined (after the usual defatting) by the procedure used for total RNA.

RESULTS

Succinic dehydrogenase (succinic-cytochrome c reductase). The level of succinic dehydrogenase activity in the whole cytoplasm was increased after hypophysectomy, this rise being reversed by growth-hormone treatment (Table 1). The level also tended to rise after adrenalectomy, the increase being almost significant ($P < 10\%$).

Most of the succinic dehydrogenase activity of the whole cytoplasm was recovered in the mitochondrial fraction, the activity of microsomal and supernatant fractions being negligible. As is evident from Table 1, mitochondrial fractions from untreated hypophysectomized rats showed an increase in activity which accounted for the increase found with the whole cytoplasm. A similar parallelism was found with adrenalectomized rats, but not with hypophysectomized rats given growth hormone, the administration of which lowered the cytoplasmic activity to normal without affecting the enhanced mitochondrial activity.

Since the cytoplasmic activity was quantitatively recovered in the mitochondrial fraction from the hypophysectomized rats given growth hormone, it is conceivable that growth hormone can reduce both the actual amount of the enzyme in the cytoplasm and the extent to which the enzyme becomes inactivated during isolation of the mitochondrial fraction. It is, however, uncertain whether inactivation is the cause of the discrepancy usually observed (Schneider & Hogeboom, 1950; Duve *et al.* 1955) between the activity of the whole cytoplasm and the activity recovered in the isolated fractions. Some evidence has now been obtained that microsomal fractions may contain an activator of the succinic dehydrogenase present in mitochondrial fractions, in accordance with previous observations by Schneider & Hogeboom (1950) and by Pressman & Lardy (1952). Until conditions can be established for the assay of this supposed activator, it is not possible to assess its role in the hormonal effects now reported.

Since the effect of hypophysectomy in raising the succinic dehydrogenase activity of the whole cytoplasm could be reversed by administration of growth hormone, it was of interest to ascertain whether the activity of cytoplasmic fractions from untreated hypophysectomized rats could be diminished by growth hormone *in vitro*. Such a diminution has in fact been observed, with concentrations of growth hormone as low as 0.1 mg./ml. (in the tissue suspension during the 'pre-incubation',

Table 1. *Succinic dehydrogenase activity in relation to hormonal status*

Activities are expressed as μ moles of succinate oxidized/min. by an amount of tissue corresponding to 100 g. body wt. In this and subsequent tables, the standard error is given after each mean difference, and the number of degrees of freedom and the probability that the difference could be due to chance are given in parentheses.

	Control rats: mean value	Experimental rats: difference from corresponding control rats		
		Hypophysectomized	Hypophysectomized + growth hormone	Adrenalectomized
Whole cytoplasmic fraction	7.09	+2.38 $\pm$ 0.965 (7; $P < 5\%$)	+0.60 $\pm$ 0.797 (5)*	+1.30 $\pm$ 0.574 (8)
Mitochondrial fraction	5.79	+2.31 $\pm$ 0.787 (7; $P < 2.5\%$)	+2.51 $\pm$ 1.10 (5)	+1.28 $\pm$ 0.734 (7)

* Difference from corresponding untreated hypophysectomized rats = -2.50 ± 0.741 (5; $P < 5\%$).

Table 2. *Nuclease activities in relation to hormonal status*

Activities are expressed as μ moles of mononucleotide liberated/min. by an amount of tissue corresponding to 100 g. body wt.; for the purpose of the calculation it is assumed (Duve *et al.* 1955) that the acid-soluble digestion products consist entirely of mononucleotides. (See also Table 1.)

		Experimental rats: difference from corresponding control rats		
	Control rats: mean value	Hypophysectomized	Hypophysectomized + growth hormone	Adrenalectomized
		Deoxyribonuclease		
Whole cytoplasmic fraction	1.51	+0.27 ± 0.100 (6; <i>P</i> < 5%)	+0.28 ± 0.064 (4; <i>P</i> < 2.5%)	+1.08 ± 0.300 (6; <i>P</i> < 2.5%)
Mitochondrial fraction	0.98	+0.23 ± 0.134 (5)	+0.56 ± 0.306 (3)	+0.44 ± 0.237 (6)
Microsomal fraction	0.12	+0.12 (0)	+0.07 (0)	+0.05 ± 0.045 (4)
Supernatant fraction	0.04	+0.05 (0)	+0.20 (0)	+0.18 ± 0.055 (4; <i>P</i> < 5%)
		Acid ribonuclease		
Whole cytoplasmic fraction	5.08	+0.74 ± 0.21 (6; <i>P</i> < 2.5%)	+2.25 ± 1.15 (4)	+1.32 ± 0.424 (6; <i>P</i> < 2.5%)
Mitochondrial fraction	3.09	-0.06 ± 0.784 (5)	+1.48 ± 0.791 (3)	+0.54 ± 0.370 (6)
Microsomal fraction	0.77	-0.06 ± 0.084 (4)	+0.17 ± 0.064 (3)*	+0.05 ± 0.100 (3)
Supernatant fraction	0.85	+0.38 ± 0.156 (4)	+0.89 ± 0.217 (3; <i>P</i> < 5%)	+0.59 ± 0.164 (3; <i>P</i> < 5%)
		Alkaline ribonuclease		
Whole cytoplasmic fraction	1.63	+0.30 ± 0.243 (6)	+0.44 ± 0.249 (4)	+0.12 ± 0.150 (6)
Mitochondrial fraction	1.14	+0.25 ± 0.160 (5)	+0.07 ± 0.253 (3)	-0.28 ± 0.257 (3)

* Difference from corresponding untreated hypophysectomized rats = +0.21 $\pm$ 0.064 (3; $P < 5\%$).

this suspension being diluted 12-fold for the actual assay). Since, however, a reduction in activity has likewise been obtained with a plasma-globulin preparation or with growth hormone subjected to treatments (heating at pH 4, or digestion with trypsin) which destroy its growth-promoting activity, the effect demonstrated with untreated growth hormone *in vitro* is apparently non-specific.

Deoxyribonuclease. As is evident from Table 2, the deoxyribonuclease activity of the whole cytoplasm showed a marked increase (71%) after adrenalectomy, and a small increase (18%) after hypophysectomy whether or not growth hormone had been administered. The activity of the mitochondrial fraction also tended to increase after hypophysectomy and, to a proportionately lesser extent, after adrenalectomy. The effect of adrenalectomy on the cytoplasmic activity was due in part to a fivefold rise in the activity of the supernatant fraction.

Acid ribonuclease. Some increase in the cytoplasmic activity occurred after adrenalectomy, and also after hypophysectomy, the effect of which was not significantly influenced by growth hormone

(Table 2). The mitochondrial and microsomal fractions showed almost normal activity in hypophysectomized rats, but the administration of growth hormone tended to increase the activity of these fractions ($P < 10\%$ and $P < 5\%$ respectively). The increase in cytoplasmic activity occurring after hypophysectomy or adrenalectomy was largely due to a rise in the activity of the supernatant fraction.

Alkaline ribonuclease. The activity of this enzyme in the whole cytoplasm and in the mitochondrial fraction was not significantly changed by hypophysectomy or adrenalectomy (Table 2).

RNA content of the supernatant fraction. It was confirmed that adrenalectomy induces a rise in the amount of RNA in the supernatant fraction (Reid, 1955, 1956), and further shown that the rise is attributable to an increase in the amount of 'polymerized' RNA (Table 3). This conclusion may also hold for hypophysectomized rats given growth hormone, the administration of which was previously shown (Reid, 1955, 1956) to induce an increase in the amount of RNA in the supernatant fraction.

Table 3. *RNA content of the supernatant fraction, in relation to hormonal status*

Values represent mg. of RNA (in the supernatant fraction)/100 g. body wt. (See also Table 1.)

	Control rats: mean value	Experimental rats: difference from corresponding control rats		
		Hypophysectomized	Hypophysectomized + growth hormone	Adrenalectomized
Total RNA	0.73	+0.01 ± 0.189 (7)	+0.08 ± 0.089 (5)	+0.13 ± 0.028 (13; $P < 0.1\%$)
'Polymerized' RNA	0.53	-0.01 ± 0.243 (7)	+0.14 ± 0.110 (5)	+0.16 ± 0.045 (13; $P < 0.5\%$)

DISCUSSION

The conclusion that the succinic dehydrogenase activity of the liver increases after hypophysectomy is in agreement with the finding of Wollman & Scow (1951) that hypophysectomized rats (consuming less food than the controls) had slightly increased succinoxidase activity, calculated as the concentration in fresh liver tissue. The observation that adrenalectomy tends to raise succinic dehydrogenase activity is also in agreement with data for succinoxidase obtained by these authors, but is in apparent disagreement with the report of Tipton, Leath, Tipton & Nixon (1946) that liver-succinoxidase activity falls after adrenalectomy. Tipton and collaborators recognize the possibility that the fall in cytochrome oxidase activity which occurs after adrenalectomy (Tipton, 1944) might render this enzyme the limiting factor in the succinoxidase system (Schneider & Potter, 1943). Should this be the case, their observation that succinoxidase activity falls after adrenalectomy could be reconciled with the present finding, although not with that of Wollman & Scow.

The effect of hypophysectomy on succinic dehydrogenase activity could evidently be due to deprivation of growth hormone and possibly also of adrenocorticotrophin, but not of thyrotrophin (Tipton *et al.* 1946; Barker, 1955). The various oxidations mediated by cytochrome *c* may be diversely affected by hypophysectomy, as is indicated by the report of Phillips & Langdon (1956) that the oxidation of reduced triphosphopyridine nucleotide is decreased, whereas that of reduced diphosphopyridine nucleotide is unaffected.

Since succinic dehydrogenase activity is increased after hypophysectomy (and possibly after adrenalectomy), whereas coenzyme A may fall after hypophysectomy (Ringler & Leonard, 1954; Bartlett, Grimmer, Beers & Shelata, 1956; but cf. Chernick & Moe, 1956), it would be of interest to know whether the catabolism occurring via the tricarboxylic acid cycle is increased, as Engel (1954) has suggested. The observation that liver slices from hypophysectomized rats have decreased respiration (Victor & Andersen, 1938; MacLeod &

Reiss, 1940; Chernick & Moe, 1956) does not disprove this suggestion, since the decrease could be due to a decrease in fatty acid oxidation. With acetate as substrate, formation of carbon dioxide by liver slices is unchanged by hypophysectomy (Baruch & Chaikoff, 1955), although an increase may occur under certain conditions after adrenalectomy (Perry & Bowen, 1956).

The apparent fall in the succinic dehydrogenase activity of the cytoplasm after treatment with growth hormone is in accord with a suggestion (Engel, 1954) that the tricarboxylic acid cycle is slowed by growth hormone, as observed under certain conditions in intact rats (Greenbaum, 1955). However, intact rats may differ from hypophysectomized rats in their response to growth hormone, as found by Ringler & Leonard (1954) and by Bartlett *et al.* (1956) in their studies on coenzyme A. The influence of hormones on the tricarboxylic acid cycle clearly warrants closer study.

The observations that deoxyribonuclease activity is markedly raised after adrenalectomy (Table 2) and that the amount of deoxyribonucleic acid in liver is increased after adrenalectomy, although not after hypophysectomy (Reid, 1956) are compatible with, but do not unequivocally support, the suggestion of Schneider & Hogeboom (1952) that liver nucleases govern the synthesis, rather than the degradation, of nucleic acids.

With ribonucleases, a synthetic function has also been suggested by Hokin & Hokin (1954), and it has been shown by Heppel, Whitfeld & Markham (1955) that pancreatic ribonuclease can in fact catalyse the synthesis of polynucleotides from cyclic nucleotides. 'Alkaline-ribonuclease activity', which has shown no dependence on hormonal status (Table 2), may depend on an enzyme which has recently been purified and which appears to be similar in specificity to pancreatic ribonuclease (Kaplan & Heppel, 1956; Roth, 1956). 'Acid-ribonuclease activity', which increases after hypophysectomy or adrenalectomy, presumably depends on a different enzyme or group of enzymes (Lamirande *et al.* 1954; Roth, 1954*a*, 1956) the specificity of which is not known. An increase in this activity would not be expected

if acid ribonuclease has a function in the synthesis of microsomal RNA, or of protein (Roth, 1954*b*), since there is evidence to suggest that the synthesis of microsomal RNA and protein is depressed by hypophysectomy or adrenalectomy (Reid, O'Neal, Stevens & Burnop, 1956). In contrast with the microsomal fraction, the supernatant fraction does show an apparent increase in the synthesis of RNA after adrenalectomy, although not after hypophysectomy. If the rise in acid-ribonuclease activity after adrenalectomy is indeed related to some change in the metabolism of the RNA of the supernatant fraction, this change might of course be an increase in RNA catabolism accompanying the apparent increase in anabolism.

In considering the data for enzyme levels in individual fractions, as distinct from the whole cytoplasm, account should be taken of a suggestion (Weiss, 1955) that an increase in the fragility of cytoplasmic particles could account for certain hormonal effects, such as the fall in the amount of mitochondrial RNA after hypophysectomy (Reid, 1955, 1956). As a possible consequence of such an increase in the fragility or permeability, there might be an apparent shift, into the microsomal or supernatant fraction, of enzymes normally located within mitochondria, as found after adrenalectomy with D-amino acid oxidase (Kuriaki & Kensler, 1954). These authors mention preliminary experiments which indicated that the proportion of cytoplasmic succinoxidase activity found in the mitochondrial fraction decreased after adrenalectomy. However, no details have been published, and no such shift is evident from the results now obtained for succinic dehydrogenase or from the data reported by Reid, O'Neal & Lewin (1956) for adenosine triphosphatase. Indeed, the data for succinic dehydrogenase indicate that the amount in the mitochondrial fraction rises after hypophysectomy and possibly after adrenalectomy, despite a fall in the yield of this fraction (Reid, 1955, 1956).

There is good evidence that deoxyribonuclease and acid ribonuclease are located in particles ('lysosomes') which are present in mitochondrial fractions but are distinct from mitochondria (Duve *et al.* 1955). Since the increases in deoxyribonuclease and acid-ribonuclease activities after adrenalectomy, and in acid-ribonuclease activity after hypophysectomy, are proportionately greater with the supernatant fraction than with the mitochondrial fraction (Table 2), it is possible that there is indeed an increase in the fragility or permeability of these particles. Such an increase could, however, scarcely account for the decrease in the yield of the mitochondrial fraction, of which these particles form only a small proportion. Previous work (Reid, 1955) suggests that the decrease is, in hypophysectomized rats, due to a decrease in RNA

and in other water-soluble constituents, the nature of which is unknown.

The results obtained for succinic dehydrogenase and adenosinetriphosphatase in the present studies support the conclusion of Greenbaum & Greenwood (1954) that the enzymes in mitochondria may, under endogenous influences, change in relative amount. The conclusion that the composition of cytoplasmic particles is not invariable also applies to the microsomal elements (Reid, 1955; Reid, O'Neal & Lewin, 1956).

SUMMARY

1. The levels of activity of succinic dehydrogenase and of nucleases in rat-liver cytoplasm have been studied in relation to alterations in hormonal balance. The distribution of activity among separated fractions (mitochondrial, microsomal and supernatant fractions) has also been studied.

2. The succinic dehydrogenase activity of the whole cytoplasm and of the mitochondrial fraction rose after hypophysectomy and tended to rise after adrenalectomy. Treatment with growth hormone reversed the effect of hypophysectomy on the whole cytoplasm.

3. Cytoplasmic deoxyribonuclease activity rose slightly after hypophysectomy (whether or not growth hormone had been given), and more markedly after adrenalectomy, the effect of which was partly attributable to a rise in the activity of the supernatant fraction.

4. The acid-ribonuclease activity of the whole cytoplasm, and that of the supernatant fraction, rose after hypophysectomy (whether or not growth hormone had been given) or after adrenalectomy.

5. Alkaline-ribonuclease activity did not alter with changes in hormonal status.

6. The increase in the amount of ribonucleic acid in the supernatant fraction which occurs after adrenalectomy was found to be due to a rise in 'polymerized' ribonucleic acid.

Thanks are expressed to Mr J. T. Nodes, B.Sc., for technical assistance, and to Miss M. A. O'Neal, M.Sc., for performing some of the hypophysectomies and for carrying out preliminary studies on enzymic assays. The authors are indebted to Professor C. H. de Duve for advice on assay methods and for the loan of a manuscript before publication. The growth hormone was donated by Armour and Co., Chicago (Dr S. L. Steelman). Samples of liver RNA were kindly provided by Mr R. H. Pain, B.A., of this Institute. The investigation was supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

REFERENCES

- Barker, S. B. (1955). *Endocrinology*, **57**, 414.
- Bartlett, P. D., Grimmett, P., Beers, L. & Shelata, S. (1956). *J. biol. Chem.* **218**, 419.
- Baruch, H. & Chaikoff, I. L. (1955). *Endocrinology*, **56**, 609.
- Chernick, S. S. & Moe, J. G. (1956). *Endocrinology*, **58**, 280.
- Duve, C. de, Pressman, B. C., Gianetto, R., Wattiaux, R. & Appelmans, F. (1955). *Biochem. J.* **60**, 604.
- Engel, F. L. (1954). *Progr. Allergy*, **4**, 227.
- Gianetto, R. & Duve, C. de (1955). *Biochem. J.* **59**, 433.
- Greenbaum, A. L. (1955). *The Hypophyseal Growth Hormone, Nature and Actions*, p. 330. Ed. by Smith, R. W., Gaebler, O. H. & Long, C. N. H. New York: McGraw-Hill Publ. Co.
- Greenbaum, A. L. & Greenwood, F. C. (1954). *Biochem. J.* **56**, 625.
- Henderson, R. W. & Rawlinson, W. A. (1956). *Biochem. J.* **62**, 21.
- Heppel, L. A., Whitfeld, P. R. & Markham, R. (1955). *Biochem. J.* **60**, 8.
- Hokin, E. & Hokin, M. R. (1954). *J. Histochem. Cytochem.* **2**, 395.
- Kaplan, H. L. & Heppel, L. A. (1956). *J. biol. Chem.* (in the Press).
- Kuff, E. L. & Schneider, W. C. (1954). *J. biol. Chem.* **206**, 677.
- Kuriaki, K. & Kensler, C. J. (1954). *J. Biochem., Tokyo*, **41**, 409.
- Lamirande, G. de, Allard, C., Costa, H. C. da, & Cantero, A. (1954). *Science*, **119**, 351.
- Lowe, C. U. (1955). *J. nat. Cancer Inst.* **15**, 1619.
- MacLeod, L. D. & Reiss, M. (1940). *Biochem. J.* **34**, 820.
- Perry, W. F. & Bowen, H. F. (1956). *Amer. J. Physiol.* **184**, 59.
- Phillips, A. H. & Langdon, R. G. (1956). *Biochim. biophys. Acta*, **19**, 380.
- Pressman, B. C. & Lardy, H. A. (1952). *J. biol. Chem.* **197**, 547.
- Reid, E. (1955). *Nature, Lond.*, **175**, 461.
- Reid, E. (1956). *J. Endocrin.* **13**, 319.
- Reid, E., O'Neal, M. A. & Lewin, I. (1956). *Biochem. J.* **64**, 730.
- Reid, E., O'Neal, M. A., Stevens, B. M. & Burnop, V. C. E. (1956). *Biochem. J.* **64**, 33.
- Ringler, I. & Leonard, S. L. (1954). *Endocrinology*, **55**, 363.
- Roth, J. S. (1954a). *J. biol. Chem.* **208**, 181.
- Roth, J. S. (1954b). *Nature, Lond.*, **174**, 129.
- Roth, J. S. (1956). *Fed. Proc.* **15**, 341.
- Schneider, W. C. & Hogeboom, G. H. (1950). *J. nat. Cancer Inst.* **10**, 969.
- Schneider, W. C. & Hogeboom, G. H. (1952). *J. biol. Chem.* **198**, 155.
- Schneider, W. C. & Potter, V. R. (1943). *J. biol. Chem.* **149**, 217.
- Tipton, S. R. (1944). *Endocrinology*, **34**, 181.
- Tipton, S. R., Leath, N. J., Tipton, I. H. & Nixon, W. L. (1946). *Amer. J. Physiol.* **145**, 693.
- Victor, J. & Andersen, D. H. (1938). *Amer. J. Physiol.* **122**, 296.
- Weiss, P. (1955). *The Hypophyseal Growth Hormone, Nature and Actions*, p. 265. Ed. by Smith, R. W., Gaebler O. H. & Long, C. N. H. New York: McGraw-Hill Publ. Co.
- Wollman, S. H. & Scow, R. O. (1951). *Endocrinology*, **49**, 105.

Studies on Ali-esterases

4. DIFFERENTIATION OF ESTERASES IN PANCREAS AND BRAIN BY THE USE OF ORGANOPHOSPHORUS INHIBITORS*

By D. K. MYERS†

Pharmaco-therapeutic Laboratory, University of Amsterdam, Amsterdam, Holland

(Received 15 March 1956)

The results obtained in previous investigations have demonstrated the value of the organophosphorus compounds as experimental tools for the differentiation of the various types of enzymes with esterase activity. By means of these inhibitors it is possible to distinguish clearly between certain of the esterases which are present in the same tissue, e.g. between the A-esterases, ali-esterases (B-type esterase) and a resistant tributyrinase which may all be present in plasma (Aldridge, 1953, 1954; Mounter & Whittaker, 1953; Myers, Schotte & Mendel, 1955a).

* Part 3: Myers, Schotte, Boer & Borsje-Bakker (1955b).

† Present address: Suffield Experimental Station, Ralston, Alberta, Canada.

Later it was also shown that at least three types of enzymes with esterase activity can be distinguished in pancreas by the use of these organophosphorus inhibitors (Myers, Schotte, Boer & Borsje-Bakker, 1955b). The lipase, which is responsible for the hydrolysis of tributyrin by pancreas suspension, is relatively resistant to inhibition by most of the organophosphorus derivatives except diethyl *p*-nitrophenyl phosphate (E 600) (cf. Aldridge, 1954); while the cholesteroesterase, which is probably responsible for the hydrolysis of methyl and phenyl butyrates by pancreas (cf. Fodor, 1950), is readily inhibited by all the organophosphorus compounds tested. The slow hydrolysis of salicyl butyrate by pancreas is due largely to a third enzyme (cf.