Inner cylinder (Fig. 2 (2) *O* and (3)). This is of ebonite, length 2·5 in., diam. 0·7874 in. (2·00 cm.), secured to the centre of a brass disk (*T*), 0·3 in. thick, diam. 3·25 in., which rests on the flange of the bearing assembly. This disk is partly cut away, to allow viewing of the liquid. Disk and cylinder were machined together, so as to ensure their being concentric and coaxial.

Head (Fig. 1 (2)). This is similar to the viscosimeter head, but consists of three horizontal plates. The lower two are fitted with rotating rings, engraved in degrees, to hold cross-wires, polarizer or quarter-wave plate.

Optical parts. A Pointolite lamp, with a 25 cm. lens, is used to project a vertical parallel beam through a hole in the bench below the apparatus. This beam is made vertical by marking a point on the ceiling vertically above the centre of the pot by plumb-line, and adjusting until the circle of light, projected through the pot, is centred on this point. The polarizer, a 5 cm. Nicol prism, is secured below the bench. The analyser (a similar prism) is placed on the middle deck of the head. For measuring angle of isocline, cross-wires, made of thin Nylon thread, are placed on the lowest deck: for measurement of birefringence, a $\frac{1}{4}$-wave plate is placed on the lowest deck. A field lens, on the top deck, allows the whole annulus of liquid to be viewed at one time. The glass plate (*Q*) and the annulus (*R*) must be free from birefringence. The hole in the annulus is just large enough to allow it to rotate freely round the inner cylinder. It was found that the annulus can be made from Perspex, 1–2 mm. thick, by cutting very lightly with a fretsaw and finishing with sandpaper.

Filling. The pot is set up in the bearing assembly: the Perspex annulus is placed in position: the inner cylinder is inserted through the annulus. The space between pot and inner cylinder is then filled through the filling hole *S* (Fig. 2 (2)) until the liquid surface reaches and wets the under side of the annulus; by filling slowly, formation of bubbles can be avoided.

Range. Velocity gradients up to 100 sec.$^{-1}$ are attainable with the present apparatus. There is no reason why higher speeds should not be used.

SUMMARY

1. A Couette viscosimeter is described, which can be made in a laboratory workshop.

2. The accuracy of measurement is 1–2 %. The range of velocity gradient is 0–75 sec.$^{-1}$.

3. Modification for measuring streaming birefringence is described.

Mr J. T. Cox was responsible for details of design and construction. We are most grateful to Dr H. G. Kuhn, of the Clarendon Laboratory, for the loan of optical parts. This work was supported by a Research grant from the Medical Research Council.

REFERENCES

Landolt-Börnstein (1927). *Physikalisch-Chemische Tabellen*, Berlin: Springer.

Lawrence, A. S. C., Needham, J. & Shen, S. C. (1944). *J. gen. Physiol.* **27**, 201.

Ogston, A. G. & Stanier, J. E. (1950). *Biochem. J.* **46**, 364.

Ogston, A. G. & Stanier, J. E. (1951). *Biochem. J.* **49**, 585.

Ogston, A. G. & Stanier, J. E. (1952). *Biochem. J.* **52**, 149.

Studies Involving Enzymic Phosphorylation

2. CHANGES IN THE HEXOKINASE ACTIVITY OF THE SMALL INTESTINE OF RATS CAUSED BY FEEDING DIFFERENT DIETS

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Although there is no universal agreement regarding the biochemical mechanisms of intestinal absorption, many workers at the present time accept the theory of Verzár & McDougall (1936), whereby the absorption of carbohydrate and fats takes place through the formation of phosphorylated intermediates. In a recent study, Lawrie & Yudkin (1949) determined the alkaline phosphatase activities of small intestine from rats fed diets containing different amounts of carbohydrate, fat and protein; they found that phosphatase activity was significantly higher in animals fed a high-protein or high-fat diet than in those fed a high-carbohydrate (balanced or fat-free) diet. This result would not have been expected on the basis of the theory that glucose and certain other monosaccharides pass from the lumen into the mucosal cells by enzymic phosphorylation, and from the cells to the tissue

spaces draining into the portal circulation by enzymic dephosphorylation; indeed it might well have been expected that the presence in the diet of large amounts of carbohydrate and the consequent need for absorption of monosaccharides would be more likely to lead to an increased activity of both phosphorylating and dephosphorylating enzymes in the intestinal mucosa.

The hexokinase activities of intestine and of other tissues from rats maintained on normal diets have recently been determined (Long, 1951). The present study is an extension of this work and deals with the hexokinase activities of the intestinal mucosa and muscle of rats fed on diets containing different proportions of carbohydrate and other constituents. It has been found that a high-carbohydrate (fat-free) diet leads, in the rat, to a significant increase in the hexokinase activity of intestinal mucosa above the normal level; conversely, a carbohydrate-free (high-fat) diet leads to a reduction in hexokinase activity of the mucosa. These variations in carbohydrate and fat content of the diet were found not to affect significantly the hexokinase activities of rat intestinal muscle.

EXPERIMENTAL

Materials

Animals. Male hooded Lister rats have been used throughout. For Exp. 1, weanling rats, 22 days old and weighing 44–62 g., were taken from the stock colony maintained by the Physiology department of this University. For Exp. 2, young adult males, weighing 197–364 g., were either taken from stock or obtained from the Rowett Institute, Bucksburn, Aberdeenshire; the previous diet of these adult rats consisted of rat cubes (purchased from the North-Eastern Agricultural Co-operative Society Ltd.).

Diets. The diets specified by Lawrie & Yudkin (1949) for their experiments were prepared and had the composition shown in Table 1.

Table 1. *Composition of diets*

(In addition to the constituents listed, all rats received two drops of cod-liver oil each week. The casein used was 'calcium caseinate' (Glaxo); dried brewer's yeast was obtained from Pharmaco-Chemical Products Ltd. The salt mixture was prepared according to Hubbell, Mendel & Wakeman (1937).)

	Diet (g./100 g.)			
	Basal	Fat-free	High-protein	High-fat
Sucrose	55	70	15	0
Lard	15	0	0	70
Casein	15	15	70	15
Dried brewer's yeast	10	10	10	10
Salt mixture	5	5	5	5

Methods

The rats were killed by decapitation and exsanguination. The small intestine was dissected out, washed and the mucosa scraped from the muscular portion, as previously described (Long, 1951). It should be emphasized that the amount of mucosa obtained in this way may be varied over a wide range. Thus in Exp. 1, for which mucosa only was required, it amounted to an average of 35% of the moist weight of the whole intestine; in Exp. 2, where a more thorough separation was needed, the weight of mucosa averaged 65% of the moist weight of the intestine.

Determination of hexokinase activity. For intestinal mucosa, a weighed portion was homogenized by grinding in a mortar with ice-cold 0·12M-potassium phosphate buffer, pH 7·8, containing 0·15M-KF (5 ml./g. tissue); for intestinal muscle, the proportions were similar but the tissue was disintegrated in an all-glass homogenizer (Potter & Elvehjem, 1936). The reaction mixture contained the following components (final concentrations in brackets): glucose (0·0024M), potassium adenosinetriphosphate (ATP) (0·005M), $MgCl_2$ (0·005M), KF (0·05M), potassium phosphate buffer, pH 7·8 (0·04M) and KCl (0·042M). Homogenate (0·20 ml., equivalent to 33 mg. fresh tissue) was added at zero time; total volume of reaction mixture, 0·50 ml. The amount of glucose disappearing in 4 min. at 30° in air was determined by the method of Nelson (1944). Control experiments in absence of ATP were always carried out simultaneously. This test system has been found to provide optimal conditions for glucose phosphorylation (Long, 1951).

Hexokinase activity has been expressed either as μg. glucose disappearing/33 mg. moist wt./4 min., as μl. glucose/mg. dry wt./hr. ($-Q_{glucose}$) or as μl. glucose/mg. defatted dry wt./hr., where 180 μg. glucose = 22·4 μl. Dry weights were obtained by drying weighed samples overnight at 105°.

Determination of alkaline phosphatase activity. A representative portion of mucosa (about 0·5 g.) was ground in a mortar with 10 ml. distilled water. A 1 ml. sample of this suspension was taken for dry weight determination; another portion was diluted tenfold with water and used for determining alkaline phosphatase activity. The reaction mixture contained the following components (final concentrations in brackets): disodium β-glycerophosphate (0·05M), $MgCl_2$ (0·01M) and veronal-carbonate buffer (King & Delory, 1940), pH 9·2 (0·0125M). At zero time the diluted enzyme suspension (2 ml.) was added and the mixture incubated at 30° for 15 min.; total volume of reaction mixture, 7·0 ml. The reaction was stopped by addition of 3 ml. 10% (w/v) trichloroacetic acid. Duplicate 4 ml. samples of filtrate were taken for determination of inorganic phosphate according to the method of Fiske & Subbarow (1925). Zero time controls and phosphate standards were always run simultaneously. This is essentially the procedure of Motzok & Wynne (1950) (see also Motzok, 1950).

Alkaline phosphatase activity has been expressed as μl. inorganic P liberated/mg. dry wt./hr. ($Q_{phosphate}$), where 31 μg. inorganic P = 22·4 μl.

Determination of fat content of tissue. Probably no method of determining the fat content of a tissue is completely free from criticism. The following procedure has been used. To 1 g. of fresh intestinal mucosa or muscle in a Pyrex boiling tube (150 × 25 mm.) were added 10 ml. $CHCl_3$ and 5 ml. methanol, and the mixture gently heated for 2–3 hr. on a water bath (bath temp. 55–60°). After cooling, the extract was filtered and the residue re-extracted in the same way. The combined filtrates were evaporated to dryness. The dry material was then extracted three times with a mixture of 2 ml. ethanol and 1 ml. diethyl ether, with gentle warming,

the extracts being filtered each time into a tared test tube. (The residue from this extraction was completely water-soluble.) The combined filtrates were evaporated to dryness and weighed.

RESULTS

The hexokinase and alkaline phosphatase activities of small intestinal mucosa of rats fed for 8 months from weaning on basal, fat-free, high-protein and high-fat diets (Exp. 1)

Six litters, each containing four male rats, were taken at weaning and divided into four groups, each group containing one animal from each litter, so as to form a randomized block design. The six rats in each particular group were housed together in a large cage and fed the appropriate diet *ad lib.* The amounts of the various diets consumed were not recorded. Body weights, determined at weekly intervals, are illustrated graphically in Fig. 1. After about 4 months, rats on the fat-free diet, having attained an average body weight of 320 g., began to lose weight and were thereafter given one drop of linseed oil each week, for it seemed possible that these animals might be suffering from a deficiency of essential fatty acids. However, after a further 2 months of relatively constant body weight, a further weight loss was noted. It is probable that lack of essential fatty acids was not solely responsible for the loss of weight, for the high-protein diet was also completely lacking in fat and the rats in this group continued to grow throughout the experiment. Rats on the basal diet grew very well and their growth was not inferior to that of stock animals on a diet of rat cubes.

Three of the animals on the fat-free diet and one on the basal diet died after about 7 months. After 8 months the remaining twenty animals were killed and determinations of hexokinase and alkaline phosphatase activities were made on the intestinal mucosa. The results obtained are shown in Table 2.

It should be mentioned here that when the hexokinase activity determinations were being planned, it was intended to assume a dry weight equal to 20·4 % of the fresh moist weight, the value found for the intestinal mucosa of rats on a stock diet (Long, 1951). For that reason, individual dry-weight determinations were not made. After the experiments were concluded, however, it did not seem justifiable to use this fixed value for percentage dry weight, in view of the widely different amounts of fat in the diet of the experimental animals. The results in Table 2 were therefore calculated in terms of fresh weight of tissue. If the dry weight of the intestinal mucosa of rats on the basal diet is assumed to be 20·4 % of the moist weight, as for animals on the stock diet, then the value for $-Q_{glucose}$ may be calculated to be 18·0 ± 1·3, which is in reasonable agreement with the

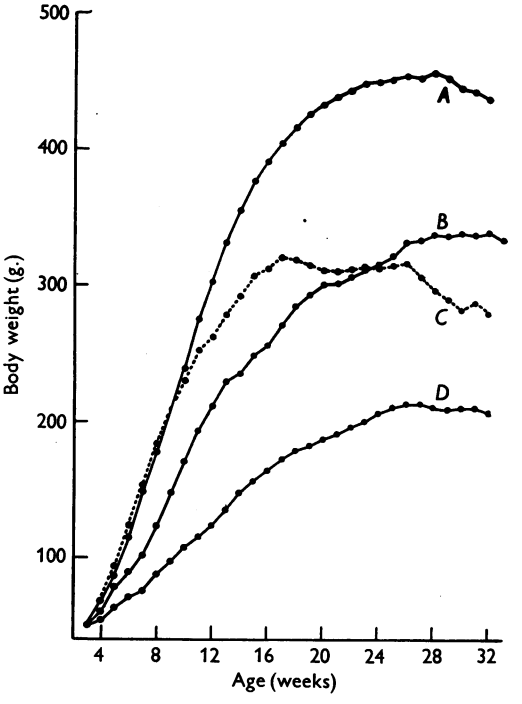

Fig. 1. Growth of weanling rats. Diets: *A*, basal; *B*, high-protein; *C*, fat-free; *D*, high-fat. Composition of diets shown in Table 1. Each point represents the mean weight of five or six rats (Exp. 1).

Table 2. *Effect of diet on hexokinase and alkaline phosphatase activity of rat intestinal mucosa (Exp. 1)*

(Weanling rats fed experimental diets for 8 months. Composition of diets given in Table 1. Values quoted are mean ± standard error of mean. Hexokinase activity expressed as μg. glucose disappearing/33 mg. moist weight/4 min.; alkaline phosphatase activity expressed as $-Q_{phosphate}$ (μl. P/mg. dry wt./hr.).)

	Diet			
	Basal	Fat-free	High-protein	High-fat
No. of animals	5	3	6	6
Body wt. (g.)	414 ± 22	283 ± 34	330 ± 7	204 ± 20
Moist wt. of whole small intestine (g.)	12·0 ± 0·9	8·1 ± 1·4	8·6 ± 0·3	7·5 ± 0·5
Moist wt. of intestinal mucosa (g.)	3·82 ± 0·46	1·93 ± 0·55	3·62 ± 0·17	3·00 ± 0·23
Hexokinase activity	65·1 ± 4·7	79·4 ± 9·7	61·3 ± 3·0	50·4 ± 2·6
Alkaline phosphatase activity	334 ± 38	323 ± 56	324 ± 43	266 ± 32

mean value of 14·8 (range 10·1–20·2) found for stock rats (Long, 1951).

The data in Table 2 have been examined statistically. It has been found, in regard to hexokinase activity, that significant differences exist between the four diets ($P < 0·001$). The fat-free diet had the largest effect, giving a result significantly higher than the basal diet ($P < 0·05$), and the high-protein or high-fat diets ($P < 0·001$). Hexokinase activities of intestinal mucosa of rats on the basal and high-protein diets were not significantly different, but both were significantly higher than for rats on the high-fat diet ($P < 0·01$ and $P < 0·05$, respectively).

Variation in alkaline phosphatase activity between the various diets was not significant.

The hexokinase activities of small-intestinal mucosa and muscle of adult rats fed for short periods on fat-free or high-fat diets (Exp. 2)

The previous experiment has shown that the nature of the diet has a significant effect on the hexokinase activity of rat intestinal mucosa. However, it might be argued that if the results had been expressed on the basis of dry weight (as were the alkaline phosphatase activities) or defatted dry weight, these effects might not have been apparent. A second experiment was therefore carried out in which the dry weight and fat content of the mucosa were determined on samples similar to those used for hexokinase activity determinations; the muscular portion of the small intestine was also investigated. This experiment was limited to two of the diets previously used, namely the high-fat and fat-free diets, for these were already known (Exp. 1) to exhibit the most marked differences.

Ten young adult male rats were divided into two groups on the basis of initial body weight (mean value, 289 g.) and fed either of the selected diets. Animals on the high-fat diet gained an average of 21 and 50 g. after 7 and 14 days, respectively; corresponding average figures for rats fed the fat-free diet were 30 and 44 g. The rats were killed after either 7 or 14 days and determinations of hexokinase activity, dry weight and fat content were carried out on intestinal mucosa and muscle. The data obtained are shown in Table 3. Since the results obtained at the end of 7 and 14 days were not significantly different, they have been combined.

The figures in Table 3 have been examined statistically. In this way it has been deduced that the hexokinase activity of intestinal mucosa is significantly higher ($P < 0·05$) for rats on a fat-free diet than for those on a high-fat diet, independently of whether the activities are expressed in terms of moist weight, dry weight or defatted dry weight of tissue. This result is of considerable interest for it means that the different levels of hexokinase activity in rat intestinal mucosa produced by feeding the fat-free or high-fat diet over a period of 8 months from weaning (Table 2) can be obtained just as readily in adult rats after feeding for 1 or 2 weeks (Table 3). The comparable values in Tables 2 and 3 for hexokinase activities based on moist weight show very close agreement.

In contrast with the result obtained for intestinal mucosa, there were no significant differences between the hexokinase activities of intestinal muscle from rats on high-fat or fat-free diets, independently of the method of expressing the results. The $-Q_{glucose}$ values (μl. glucose/mg. dry wt./hr.) of $10·3 \pm 2·0$ and $7·6 \pm 1·5$ agree satisfactorily with the average figure of 11·8 (range 9·7–13·9) found for rats on the stock diet (Long, 1951).

DISCUSSION

Although an exact comparison cannot be made, two of the results presented in this paper seem to be at variance with the findings of Lawrie & Yudkin (1949). First, the growth rates of weanling rats on the various diets are completely different in the two investigations. Those obtained in the present work depended markedly upon the composition of the diet, rats in the basal group attaining a maximum weight of about 450 g., while those in the high-fat group reached only half this value. Lawrie & Yudkin (1949), on the other hand, found their male

Table 3. *Hexokinase activities of small-intestinal mucosa and muscle of rats fed a high-fat or fat-free diet for 7 or 14 days (Exp. 2)*

(Five young adult male rats in each group, paired according to initial body weight. Composition of diets given in Table 1. Values quoted are mean ± standard error of mean.)

	Mucosa		Muscle	
	Fat-free diet	High-fat diet	Fat-free diet	High-fat diet
Moist wt. of tissue (g.)	5·34 ± 0·23	6·50 ± 0·50	3·55 ± 0·13	3·34 ± 0·02
Dry wt. (% wet wt.)	20·8 ± 1·3	24·7 ± 2·8	20·9 ± 1·2	21·0 ± 0·8
Fat content (% wet wt.)	9·5 ± 0·9	10·6 ± 0·4	4·0 ± 0·4	4·4 ± 0·4
Hexokinase activity expressed as:				
a, μg. glucose/33 mg. moist wt./4 min.	71·9 ± 9·1	45·1 ± 2·4	37·3 ± 6·1	27·7 ± 5·6
b, μl. glucose/mg. dry wt./hr.	20·1 ± 3·4	10·8 ± 1·2	10·3 ± 2·0	7·6 ± 1·5
c, μl. glucose/mg. defatted dry wt./hr.	38·2 ± 6·8	20·2 ± 3·3	12·8 ± 2·3	9·5 ± 2·1

rats to attain a maximum weight of only 202 g., with no significant variation between the groups on different diets. It does not seem possible to attribute this lack of agreement to the fact that different strains of rats were employed, for males of the Hartwell strain used by Lawrie & Yudkin (1949) are well known to attain a body weight of about 420 g. at 6 months when fed an adequate diet (Hartwell, 1926) and are thus quite comparable with the hooded Lister rats used in the present work. One explanation of this discrepancy might be that Lawrie & Yudkin (1949), when preparing their diets, used rather highly purified materials deficient in certain essentials, so that even rats on the basal (balanced) diet grew no faster than those on the high-fat diet. On general grounds, rats on a balanced diet would be expected to grow more rapidly than those on a 70% fat diet, for animals in the latter group would satisfy their caloric needs without receiving enough protein and perhaps other nutrients for maximal rate of growth. A difference in growth rate similar to that found in the present work has also been noted by Lutwak-Mann (1952).

The values recorded for the alkaline phosphatase activities of intestinal mucosa (Table 2) show no significant differences due to variation of diet. This result also differs from that claimed by Lawrie & Yudkin (1949), who found the alkaline phosphatase activity of whole intestine to be significantly higher in rats fed a high-protein or high-fat diet than in those fed the basal or fat-free diets. These different observations, of course, are not necessarily inconsistent for they refer to mucosa and whole intestine respectively. In addition, there were considerable differences in the methods used for estimating enzymic activity and finally the effect of strain difference of rats cannot be excluded here.

The main results reported in this paper establish a clear dependence of hexokinase activity of rat intestinal mucosa upon the composition of the diet. It is noteworthy that the very significant difference between the hexokinase activities of intestinal mucosa of rats fed for 8 months from weaning on high-fat and fat-free diets could be almost exactly duplicated in experiments with adult animals after only 1 or 2 weeks on these diets (Tables 2 and 3). The differences cannot be explained on the basis of variation of dry weight or defatted dry weight of the mucosae and appears to be equally real no matter what the basis for comparison. An interesting result from Exp. 1 is the finding that while there is a general positive correlation between the proportion of carbohydrate in the diet and the hexokinase activity, this correlation is by no means absolute for there was no significant difference between the hexokinase activities of intestinal mucosa of rats on the basal and high-protein diets, although the carbohydrate contents were 55 and 15% respectively.

In an earlier paper (Long, 1951), it was calculated that the hexokinase activity of rat intestinal mucosa could account for only about 30% of the experimentally determined rate of glucose absorption. However, this result did not exclude a less important role for hexokinase in the absorptive process. If a relationship does indeed exist between rate of absorption and the activity of phosphorylating enzymes in the intestine, then the present work may recall the observations of Westenbrink (1934), who found that the rate of intestinal absorption of individual monosaccharides was considerably increased by prior feeding of the appropriate monosaccharide, even for only a few days. Further, the results obtained here might also be taken to support the theory that absorption is related solely to the enzymic activity of the mucosa, for no parallel changes in the hexokinase activity of intestinal muscle have been observed.

SUMMARY

1. The hexokinase activities of intestinal-mucosa homogenates from rats fed for 8 months from weaning on different diets have been determined. Highest activity was found in rats fed a fat-free diet and lowest in those fed a high-fat diet. Intermediate values were obtained in rats fed basal (balanced) and high-protein diets. Alkaline phosphatase activities of intestinal mucosa showed no significant differences.

2. Rats fed for only 1 or 2 weeks on high-fat and fat-free diets also showed statistical differences in the hexokinase activities of their intestinal mucosae. No differences were found between hexokinase activities of intestinal muscle of the two groups.

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REFERENCES

Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
Hartwell, G. A. (1926). *Biochem. J.* **20**, 751.
Hubbell, R. B., Mendel, L. B. & Wakeman, A. J. (1937). *J. Nutrit.* **14**, 273.
King, E. J. & Delory, G. E. (1940). *Enzymologia*, **8**, 278.
Lawrie, N. R. & Yudkin, J. (1949). *Biochem. J.* **45**, 438.
Long, C. (1951). *Biochem. J.* **50**, 407.
Lutwak-Mann, C. (1952). *Biochem. J.* **52**, 356.
Motzok, I. (1950). *Biochem. J.* **47**, 193.
Motzok, I. & Wynne, A. M. (1950). *Biochem. J.* **47**, 187.
Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
Potter, V. R. & Elvehjem, C. A. (1936). *J. biol. Chem.* **114**, 495.
Verzár, F. & McDougall, E. J. (1936). *Absorption from the Intestine.* London: Longmans Green and Co.
Westenbrink, H. G. K. (1934). *Arch. néer. Physiol.* **19**, 563.

The Sulphatase of Ox Liver

1. THE COMPLEX NATURE OF THE ENZYME

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The enzyme sulphatase present in many animal tissues (Fromageot, 1938) has up to the present been little studied in comparison with the corresponding enzyme of *Aspergillus oryzae*. In view of the importance of sulphuric acid esters in many metabolic processes in the animal body it appeared that the sulphatase of animal tissues might be worthy of a detailed study. The method of assay used in the present work was based on that of Robinson, Smith & Williams (1951) which utilizes potassium 2-hydroxy-5-nitrophenyl sulphate (nitrocatechol sulphate) as substrate, and follows the sulphatase activity by the colorimetric estimation of the liberated 4-nitrocatechol. This method is not applicable to whole homogenates of animal tissues, especially liver, as such homogenates are apparently capable of metabolizing nitrocatechol, a fact which has recently been noted by Dodgson, Spencer & Thomas (1952). The method is, however, suitable for the assay of sulphatase in the partly purified preparations used in the present investigations, as under such conditions the recovery of added nitrocatechol is quantitative.

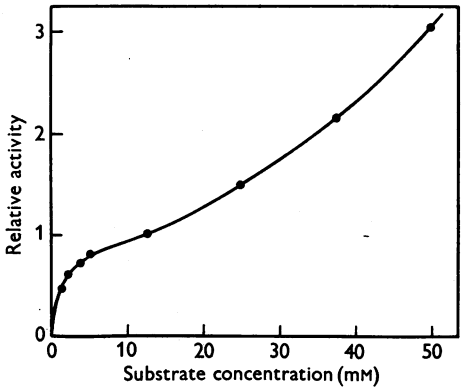

Fig. 1. Effect of varying substrate concentrations on reaction velocity. Final volume of reaction mixture 0·8 ml. containing 0·2 ml. unfractionated sulphatase preparation. Incubated 1 hr. at 37° in 0·05 M-citrate buffer, pH 6·0.

Preliminary investigations using this substrate showed that crude sulphatase preparations gave a very anomalous substrate concentration-reaction velocity curve (Fig. 1) and it appeared that more than one enzyme might be present. This paper describes the preparation of two distinct sulphatase fractions from such crude solutions.

METHODS

Preparation of substrate

The method used was essentially that of Smith (1951), except that it was found more convenient to prepare the substrate as the dipotassium 2-hydroxy-5-nitrophenyl sulphate instead of the monopotassium salt.

To 70 g. KOH and 70 g. potassium persulphate dissolved in 1 l. water were added 30 g. *p*-nitrophenol. The reaction mixture was left 48 hr. at 37°, then acidified to pH 4 with H_2SO_4, and free phenols extracted with ether. The aqueous solution was then made strongly alkaline to litmus with KOH and concentrated *in vacuo* to about 300 ml. The solution, and any precipitate, was poured into 2 vol. acetone and the mixture filtered. After washing the residue with acetone-water (2:1, v/v), the combined filtrates were taken to dryness *in vacuo* and the residue recrystallized three times from water. Bright yellow crystals of dipotassium 2-hydroxy-5-nitrophenyl sulphate dihydrate were obtained with a yield