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β-Glucuronidase and Cell Proliferation*

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After repeated feeding of menthol to mice, Fishman (1940) obtained results which, on statistical examination, showed an increase in β-glucuronidase activity in liver, spleen and kidney, as compared with organs from untreated animals. Similar results were obtained in dogs fed with borneol. Glucuronidase in uterus and other sex organs was unaffected by menthol and borneol. In Fishman's own interpretation of these important experiments, β-glucuronidase is assumed to be responsible for glucuronide synthesis in the body. A synthetic role for the enzyme has, however, still to be demonstrated, its physical properties and distribution in the body having been studied solely by means of its hydrolytic action on conjugated glucuronides. Since menthol and borneol have been proved to be excreted as the glucuronides in, e.g. the dog, and may conceivably behave in the same way in the mouse, Fishman suggested that in his experiments he was measuring adaptation by glucuronidase in response to the presence of excess substrate for its hypothetical synthetic action. Later this theory was extended to explain the elevation in uterine glucuronidase observed after administration of oestrogens to ovariectomized mice (Fishman & Fishman, 1944; Fishman, 1947). Oestrogens did not affect the enzyme in liver, spleen and kidney, and the additional assumption was required, and made, that the enzyme is specific in its synthetic action, according to its source, for different groups of substrate. No such specificity was, however, observed in its hydrolytic action *in vitro*, menthol glucuronide being used throughout in the assay of uterine glucuronidase under conditions found to be optimal for hydrolysis by spleen preparations.

Fishman determined the activity of his enzyme extracts by measuring, by means of its reducing power, glucuronic acid liberated from menthol glucuronide (Fishman, 1939). Sources of error in this procedure, arising largely from its lack of specificity, have been pointed out by other authors (Graham, 1946; Levvy, 1946, 1948), and have led to the development of more satisfactory methods of assay (Talalay, Fishman & Huggins, 1946; Kerr, Graham & Levvy, 1948).

Using phenol glucuronide as substrate in the assay of glucuronidase (Kerr *et al.* 1948), an attempt was made to confirm Fishman's findings (1940) with menthol. Within 24 hr. of a single intraperitoneal injection of L-menthol into mice, there was a marked rise in glucuronidase activity in liver, but not in spleen and kidney. Liver damage was observed and confirmed histologically, and it was subsequently shown that a rise in β-glucuronidase in liver or kidney, depending upon the organ or organs attacked, followed administration of a variety of toxic agents to mice. A more extensive examination of the action of menthol revealed, in addition to the effect on liver, delayed damage to kidney, followed by an increase in glucuronidase activity in this organ also. An increase in the glucuronidase activity of an organ was found, in general, to be associated with active cell proliferation provoked by injury, rather than with the injury itself, and high values were seen in the livers of adult mice after sub-total hepatectomy, and in the liver, spleen and kidneys of infant mice.

* Preliminary accounts of parts of this work have been published elsewhere (Kerr & Levvy, 1947; Kerr, Levvy & Campbell, 1947), and the principal findings were described in a paper read to the Biochemical Society on 27 September 1947 (Levvy, Kerr & Campbell, 1948).

EXPERIMENTAL AND RESULTS

Enzyme assay. In the assay of kidney glucuronidase it was assumed that the conditions for optimum hydrolysis of phenol β-D-glucuronide would be the same as those previously found to hold for spleen and liver preparations (Kerr *et al.* 1948). All preparations of the enzyme were diluted to final volumes giving readings of 20–40 μg. phenol in the assay, after correction for blanks. The results are shown in the tables and figures in terms of glucuronidase units (G.U.)/g. moist tissue, where 1 G.U. liberates 1 μg. phenol in 1 hr. from 0·015 M-phenol glucuronide at 38° and pH 5·2. The standard error is given wherever possible. Although frequently based upon too small a group of animals to have any statistical value, it shows the variation in the individual figures in a convenient form.

Histology. Portions of organs from animals used for enzyme assay, or whole organs from other animals treated similarly, were fixed immediately in Susa and taken in the usual way through the ethanols to a mixture of chloroform and cedarwood oil, and finally cleared in pure cedarwood oil. After embedding in paraffin wax, sections were cut at 8 μ. and stained with Mayer's haematoxylin and eosin. The distribution of fat was studied in frozen sections, prepared from tissues rapidly fixed by heat in formol saline, and stained with haematoxylin and Sudan III.

Damage, repair and cell division are shown in the tables by an arbitrary system of + signs. In the case of damage, + indicates that while definite it was neither severe nor extensive, and + + + that it was at its greatest for the toxic agent in question. The course of repair is measured likewise, + + + indicating that replacement of damaged tissue by normal cells is practically complete. Under cell division, an estimate is given of the number of mitotic and amitotic figures and hyperchromatic nuclei *in excess of normal.* No histological findings are given for spleen since deviations from normal could never be distinguished in this organ.

Normal mice and vehicle controls. Average values for β-glucuronidase in each organ were the same for normal adult mice (30–40 g.) of both sexes and drawn from three different colonies, and all the results are grouped together in the tables. Spleen showed greater variation in its normal glucuronidase activity than did liver or kidney. Intraperitoneal injection of relatively large volumes of 0·9 % sodium chloride solution, olive oil or nut oil (the vehicles used for administration of toxic agents) had no effect on glucuronidase in any of the three organs examined after an interval of 1–2 days. These results are not shown. The relatively small number of experiments in which nut oil was used as a vehicle, olive oil being unobtainable, are included in the tables with those done with the latter as medium.

Effects produced by a single injection of L-menthol. Intraperitoneal injection of L-menthol (Table 1) caused a rapid rise in liver glucuronidase activity, reaching a maximum after 24 hr. and persisting for 7 days. Greatest liver damage was observed after 24 hr., but repair processes were not perceptible at this time. After 14 days, repair was almost complete and the enzyme level had returned to its original value, although cell division still seemed to be slightly in excess of normal. In the first 24–48 hr., kidney was normal in structure and in its enzyme activity, but after 3 days damage was evident and the figure for glucuronidase had risen after 7 days. At the end of 14 days this organ was normal in all respects. No effect of menthol on spleen glucuronidase was observed at any stage. Sex did not influence the results obtained with liver and kidney.

Mills (1947) found beef spleen glucuronidase to consist of two fractions with slightly different pH optima for the hydrolysis of menthol glucuronide, and the pH activity curves for hydrolysis of phenol glucuronide by enzyme from mouse spleen and liver (Kerr *et al.* 1948) had subsidiary peaks at pH 4·5. An experiment was done in which spleen, liver and kidney glucuronidase activities, 24 hr. after injection of menthol, were compared with normal at pH 4·5 instead of 5·2. The change in pH had no appreciable effect on the results compared with those shown in Table 1.

No details of the toxic action of menthol could be found in the literature. A brief description of the changes seen in liver and kidney may be of interest. In the liver, the first deviation from normal was cloudy swelling, followed by fatty change and necrosis surrounding the central vein and extending about a third of the way into the lobule. The nuclei showed hypertrophy and hyperchromatism, many

Table 1. *Changes in β-glucuronidase and histological findings after injection of mice with L-menthol*

(333 mg. Menthol/kg. injected intraperitoneally in olive oil. Average enzyme activity and standard error expressed as G.U./g. moist tissue (see text). Number of animals in group shown by figures in brackets.)

		Liver				Kidney			
Interval (days)	Spleen enzyme	Enzyme	Damage	Cell division	Repair	Enzyme	Damage	Cell division	Repair
Untreated	636±70 (23)	273±13 (23)	—	—	—	363±24 (11)	—	—	—
0·125–0·5	720±63 (9)	467±24 (9)	—	—	—	381±40 (9)	—	—	—
1	690±41 (3)	823±135 (3)	+ + +	0	0	285±46 (3)	0	0	0
2	738±86 (3)	884±74 (6)	—	—	—	344±71 (3)	—	—	—
3	903±208 (3)	953±39 (3)	+ +	+ + +	+	—	+ +	0	0
7	646±86 (6)	775±46 (7)	+	+ +	+ +	603±52 (7)	0	+	+ + +
14	600±14 (3)	318±17 (3)	+	+	+ +	337±23 (3)	0	0	+ + +

binucleate cells appeared (amitotic division), and at a later stage mitotic division became evident. The Küpffer endothelial cells were swollen. In the case of kidney, the damage was not severe, being confined to the distal portions of the convoluted tubules and to some glomeruli, the endothelium of which was swollen and in places necrotic. Intraperitoneal injection of mice with large doses of menthol (about 0·7 g./kg.) caused prolonged depression of the respiration and unconsciousness. No attempt was made to determine the lethal dose.

Repeated administration of L-*menthol and* L-*menthol β*-D-*glucuronide*. Results for β-glucuronidase in spleen, liver and kidney, after intraperitoneal injection of mice with L-menthol or its glucuronide twice or thrice daily for varying periods (Table 2), were similar to those obtained after a single injection of L-menthol. The glucuronide was injected as a neutral solution in 0·9 % sodium chloride solution and menthol itself as a solution in olive oil. (For the preparation of neutral solutions of acid compounds in 0·9 % sodium chloride solution, see Chance, Crawford & Levvy, 1945.) Repeated oral administration (Odell, Skill & Marrian, 1937) of menthol produced an increase in liver glucuronidase activity of the same order as the injections, except in mice receiving a total dose of 9·3 g./kg. in which the rise was barely perceptible. The latter was, however, as great as that obtained by Fishman (1940) and proved to be statistically significant ($P = 0{\cdot}01$). This experiment was carried out exactly as described by Fishman except that three of the mice were given a solution of menthol in olive oil instead of an emulsion in soap solution. The change in vehicle had no effect on the response of the enzyme, and only solutions in oil were used in the other feeding experiments (total dose 1·2 and 2·0 g./kg.). No histology was done in the experiments listed in Table 2, except in the case of menthol glucuronide, which produced changes similar to those seen after a single injection of menthol.

Table 2. *Changes in β-glucuronidase after repeated administration of* L-*menthol and* L-*menthol β*-D-*glucuronide*

(Results expressed as in Table 1)

Agent and mode of administration	Total dose (g./kg.)	Interval after 1st administration (days)	Average enzyme activity and S.E. (G.U./g. moist tissue) Spleen	Liver	Kidney
Untreated	—	—	636±70 (23)	273±13 (23)	363±24 (11)
L-Menthol, orally	1·2	3	843±185 (3)	741±146 (3)	—
	2·0	1	—	895±77 (6)	—
	9·3	5	499±48 (6)	369±40 (6)	260±2 (2)
L-Menthol, intraperitoneally	0·8	2	254±38 (3)	1149±136 (3)	—
	1·2	3	599±42 (6)	869±58 (6)	—
L-Menthol β-D-glucuronide, intraperitoneally	2·3	1·5	995±95 (3)	1104±222 (3)	295±13 (3)

Changes produced by single injections of a variety of substances. The changes in β-glucuronidase activity and histological findings in liver and kidney after injection of various substances, some of them known liver or kidney poisons, are summarized in Table 3. Spleen was also examined in these experiments. Since any changes in glucuronidase in this organ were relatively small, with wide variation in individual figures, the results are not shown.

Subcutaneous injection of carbon tetrachloride in olive oil caused severe fatty degeneration and early necrosis in liver within 24 hr. After 3 days, damage was extensive, but repair processes had commenced, and after 7 days repair was far advanced. A marked increase in liver glucuronidase activity occurred within 24 hr., and this was maintained for 7 days. There were no marked pathological changes in kidney at any stage, nor was there any rise in glucuronidase in this organ. Intraperitoneal injection of carbon tetrachloride (0·5–2 g./kg.) produced a change in liver glucuronidase similar to that already described for subcutaneous injection.

Mercuric nitrate given subcutaneously in 0·9 % sodium chloride solution had no very marked effect on liver, but produced severe cortical necrosis with hyaline casts in kidney within 24 hr. Kidney glucuronidase activity showed no rise at this stage, but after 3 days, by which time repair was practically complete, it was more than twice its normal value.

The changes in liver after subcutaneous injection of chloroform in olive oil resembled those produced by carbon tetrachloride. Kidney, however, showed an interesting sex specificity in the response of the enzyme to chloroform. In agreement with the observation of Eschenbrenner (1944), this compound was found to cause renal necrosis in male, but not in female mice. The rise in kidney glucuronidase activity, which was confined to male mice, was not seen in the early stages of the damage, but was evident after 8 days, by which time repair was extensive. An increase in liver glucuronidase

Table 3. *Effects of various agents on liver and kidney*

(Results expressed as in Table 1)

Agent and dose	Interval (days)	Sex	Liver				Kidney			
			Enzyme	Damage	Cell division	Repair	Enzyme	Damage	Cell division	Repair
None	—	♀, ♂	273±13 (23)	—	—	—	363±24 (11)	—	—	—
Carbon tetrachloride	1	♂	1138±148 (3)	+ + +	0	0	139±9 (3)	+ (?)	0	0
(5·3 g./kg.)	7	♂	927±48 (4)	+	+ + +	+ +	323±45 (4)	0	0	0
$Hg(NO_3)_2$	1	♂	436±28 (2)	+	0	0	208±42 (3)	+ + +	0	0
(20 mg./kg.)	3	♂	469±29 (5)	0	+	0	808±61 (5)	0	+	+ + +
Chloroform	1	♂	939±90 (7)	+ + +	+ +	0	194±16 (6)	+ + +	0	0
(2 g./kg.)	8	♂	711±34 (3)	+	+ +	+ +	628±119 (3)	+	+ +	+ +
	1	♀	583±27* (3)	—	—	—	251±18 (3)	0	0	0
	8	♀	608±44 (3)	+	+ +	+ +	274±15 (3)	0	0	0
Yellow phosphorus	2	♂	91±23 (3)	+ + +	0	0	338±35 (3)	+ (?)	0	0
	5	♂	744±37 (3)	+ +	+ +	+	462±89 (3)	0	0	0
(7·5 mg./kg.)	10	♂	429±80 (3)	0	0	+ + +	309±32 (3)	0	0	0
Sulphathiazole (43 g./kg.)	3	♂	460±29 (3)	+	0	0	368±13 (3)	0	0	0
Pregnanediol (333 mg./kg.)	1·7	♀	287±65 (3)	0	0	0	321±64 (3)	0	0	0
†Pregnanediol β-D-glucuronide (800 mg./kg.)	1·7	♀	241±36 (3)	—	—	—	264±19 (3)	—	—	—
Ether (40 min. deep anaesthesia)	1·75	♀, ♂	265±30 (4)	0	0	0	309±63 (3)	0	0	0
Sodium sulphapyridine monohydrate (18–36 g./kg.)	2	♀, ♂	327±17 (7)	+ +	+	+	362±11 (7)	+ (?)	0	0

* Results for glucuronidase in liver obtained after intraperitoneal injection of 0·5 g. chloroform/kg.

† Pregnane-3(α):20(α)-diol glucuronidic acid free from pregnane-3(α)-ol-20-one glucuronidic acid (Sutherland & Marrian, 1947).

activity was observed after injection of as little as 0·2 g. chloroform/kg. subcutaneously.

Yellow phosphorus, injected subcutaneously in olive oil, had no marked effect on kidney, but produced profound and extensive changes in liver (congestion, fatty degeneration and necrosis). From the results of experiments dealt with above it will have been noted that there may be no rise in glucuronidase activity in an organ when damage is at its height. In the case of phosphorus, there was an unmistakable initial drop in the activity of the enzyme, to one-third of its normal value. When, at the end of 5 days, repair was well under way, the enzyme level showed the usual increase, only to fall again when repair was complete.

Of the remaining substances listed in Table 3, ether and pregnanediol produced no pathological changes and had no effects on glucuronidase in either liver or kidney. Pregnanediol glucuronide resembled the parent compound in its effects on the enzyme, but was not examined for histological effects. Ether was given by inhalation, and pregnanediol and its glucuronide were injected intraperitoneally as suspensions in olive oil. Sulphathiazole caused cloudy swelling in liver after subcutaneous injection of a very large dose as a neutral solution in 0·9% sodium chloride solution. There was a small, but significant ($P=0·05–0·02$) rise in liver glucuronidase activity. This compound had no effect of any kind on kidney. Sodium sulphapyridine, given in the same way as sulphathiazole, caused fatty degeneration and necrosis in liver, accompanied by some cell proliferation, but without appreciable change in the enzyme level. In some animals there was slight damage to the kidneys, again without any rise in glucuronidase activity.

Uranyl acetate. Results for glucuronidase in liver and kidney after subcutaneous injection of mice with varying doses of uranyl acetate in 0·9% sodium chloride solution (Fig. 1) illustrate the point that increasing the dose of a toxic agent may retard the rise in glucuronidase activity in the early stages of poisoning, and may even cause an initial drop in the enzyme level. Each point in the figure is an average for a group of three male mice, killed 2 days after injection. Severe tubular 'nephrosis' was noted at this stage with all four doses of the toxic agent. Cell proliferation could also be seen after all but the

largest dose, becoming more marked as the dose fell. In the case of liver, the histological findings were more difficult to interpret as the damage, which was mainly subcapsular, was transitory and rapidly succeeded by intensely active cell proliferation. Only the latter response was observed after injection of the smallest dose of uranyl acetate. In general, however, damage was greater and repair processes slower to appear as the dose was increased. Ten days after injection of the smallest dose of uranyl acetate repair was finished in both liver and kidney, and their enzyme levels had returned to normal. No change in spleen glucuronidase was produced by uranyl acetate.

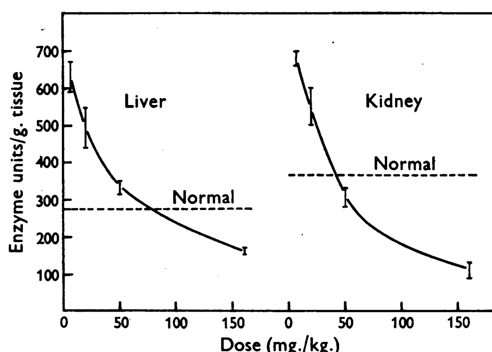

Fig. 1. Liver and kidney glucuronidase activity 2 days after subcutaneous injection of mice with varying doses of uranyl acetate. Mean ± S.E. shown for each point.

The possibility that in a severely damaged organ an apparently normal value for glucuronidase may be observed at a certain stage, even though cell proliferation may have commenced, probably explains the fact that only small rises in the liver enzyme were observed after prolonged feeding of menthol (total dose 9·3 g./kg., Table 2) or subcutaneous injection of sodium sulphapyridine (Table 3).

Phenylarsenoxide. Results obtained with this compound (Fig. 2) show the changes in liver and kidney glucuronidase activity at various stages in different degrees of poisoning. Phenylarsenoxide was injected subcutaneously as a neutral solution in 0·9 % sodium chloride solution. Each point in the figure is an average for a group of three male mice, except in the case of the 1 day figures with the larger dose, which are both based on six results. Phenylarsenoxide caused peripheral lobular necrosis and fatty degeneration in liver, and diffuse nephritis in kidney. Damage to both organs was intense 1 day after injection of 1 mg./kg., with no signs of repair. After 3 days, repair processes had become evident, and replacement of damaged tissue was well advanced after 5 days. At the end of 10 days, kidney was entirely normal and liver repair was almost complete. With the smaller dose (0·2 mg./kg.), cell proliferation was marked in both organs after 1 day, and repair was far advanced after 4 days. Phenylarsenoxide had no effect on spleen glucuronidase, and the histological changes produced in liver and kidney were similar for both sexes.

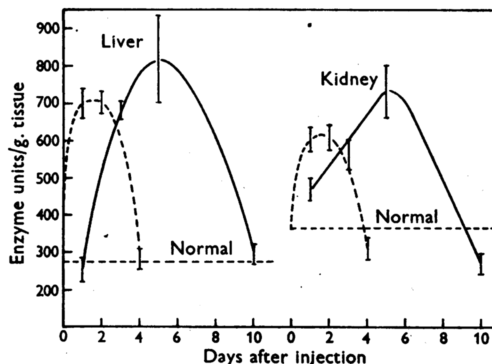

Fig. 2. Liver and kidney glucuronidase activity at varying periods after subcutaneous injection of mice with phenylarsenoxide. ——, 1 mg./kg.; – – –, 0·2 mg./kg. Mean ± S.E. shown for each point.

Effects of various substances on the enzyme in vitro. All the substances examined for their effects on β-glucuronidase activity *in vivo*, with the exception of pregnanediol glucuronide, were tested for their effect on the assay *in vitro* in a concentration of 0·1 % (w/v). A solution in the medium used for injection was added to the citrate buffer and shaken vigorously. In no case did the presence of the agent in the incubation mixture affect the activity of β-glucuronidase from normal mice.

Infant mice and partially hepatectomized mice. As shown in Table 4, glucuronidase activity in spleen, liver and kidney was much higher in young mice, ranging in age from 1 to 15 days, than in normal adults. The remaining lobes of liver in adult mice (male and female), 3–8 days after sub-total hepatectomy, were hypertrophied. The glucuronidase level was high and cell proliferation was very active. In preparing the animals, 60 % of the liver was removed by cautery or ligature under ether anaesthesia, the whole operation taking less than 10 min. There was no difference in the final result between the alternative surgical techniques, nor at the various times of examination. One animal (result omitted from Table 4) was comatose and apparently about to expire when killed 3 days after operation. As expected, the remaining fraction of the liver showed no increase in weight, in the glucuronidase activity nor in cell proliferation.

Table 4. *β-Glucuronidase after sub-total hepatectomy and in infant mice*

(Results expressed as in Table 1)

Age (days)	Treatment	Average enzyme activity and S.E. (G.U./g. moist tissue) Spleen	Liver	Kidney
Adult	None	636±70 (23)	273±13 (23)	363±24 (11)
1*	None	5100 (3)	1370 (3)	881 (3)
5*	None	2670 (2)	1294 (2)	702 (2)
5*	None	3820 (2)	1432 (2)	883 (2)
13*	None	1521 (2)	2218 (2)	606 (2)
15	None	5169±2820 (3)	1239±49 (3)	727±108 (3)
Adult	Partial hepatectomy 3–8 days previously	—	1046±88 (10)	—

* Each organ pooled before enzyme assay.

DISCUSSION

Our own results with menthol confirm Fishman's (1940) findings in so far as the enzyme in liver and kidney is concerned. It seems, however, that Fishman suppressed repair processes by overdosage with menthol, and thus obtained a rise in glucuronidase which was only a small fraction of that provoked by the first doses of the compound. No explanation can be offered for our failure to observe the rise in the activity of spleen glucuronidase in menthol-treated mice reported by Fishman, unless the discrepancy has its origin in the wide variation in its glucuronidase level normally shown by spleen. This variation one might expect if glucuronidase activity is a measure of the amount of cell proliferation in progress.

The present work shows that the effects of menthol on β-glucuronidase activity *in vivo* bear no relation to its glucuronidogenic property, but are secondary to its hitherto unsuspected toxic action on liver and kidney. It seems impossible that chloroform, carbon tetrachloride, mercuric nitrate, phosphorus or uranyl acetate should give rise to a glucuronide in the body, and yet all these substances have been found to cause striking changes in glucuronidase activity. Of other substances which caused a rise in glucuronidase, phenylarsenoxide could conceivably form a derivative conjugated with glucuronic acid, and evidence has been obtained that sulphathiazole is partially excreted in rabbits as the glucuronide of a hydroxy derivative (Thorpe & Williams, 1940). In spite of the very large dose injected, sulphathiazole caused only a relatively small rise in glucuronidase activity in mice, and this was confined to liver. No change in glucuronidase was observed after injection of two compounds which are known to be glucuronidogenic, pregnanediol (Venning & Browne, 1936) and sodium sulphapyridine (Scudi, 1944). It should be pointed out that a change in experimental conditions might reveal an effect of sulphapyridine on glucuronidase in liver, since it produced some damage in this organ. The effect of menthol glucuronide on liver glucuronidase was due presumably to menthol liberated by the enzyme initially present. Fishman's theory (see p. 462) provides no explanation for a change in the enzyme brought about by administration of a compound already conjugated with glucuronic acid.

On the basis of the experiments described above it is not possible to decide whether β-glucuronidase is actually concerned in cell proliferation, or whether the increases in activity observed merely reflect an increase in metabolic activity. It is interesting to note, however, that the rise in the enzyme level occasionally slightly preceded the first appearance of cell division which was definitely in excess of normal. Whatever the cause of the parallelism between the glucuronidase activity in an organ and the amount of tissue growth in progress, it provides a straightforward explanation of the changes in the enzyme in liver and kidney which follow administration of menthol and other substances to mice. It seems possible that the same explanation can be applied to the effect of oestrogens on uterine glucuronidase (Fishman & Fishman, 1944; Fishman, 1947), and to a recent observation (Fishman & Anlyan, 1947), which suggests that in some cases of human carcinoma the tumour contained more glucuronidase than the corresponding normal tissue. The possible bearing of our results with carbon tetrachloride on the finding (Pincus & Martin, 1940) that, in liver poisoning produced by this compound, the physiological activity of oestrone is enhanced is of interest.

It is no longer necessary to speculate on the probable role of glucuronidase in the body in order to explain the changes in activity produced by extrinsic agents. The citation by Fishman (1947) of the work of other authors in support of his contention that the enzyme acts synthetically, however, makes it necessary to consider their results from this angle. Florkin, Crismer, Duchateau & Houet (1942) obtained evidence for the condensation of glucuronic acid with borneol in the presence of β-glucuronidase, but the percentage conjugation was very small under extreme conditions, and they concluded: 'Quant à

savoir si cette synthèse enzymatique correspond au mécanisme réalisé *in vivo*, c'est évidemment une autre affaire.' In the work of Lipschitz & Bueding (1939) and Crépy (1946) on the formation of conjugated glucuronides by surviving liver slices, there is no suggestion that the enzyme concerned is β-glucuronidase. De Meio & Arnolt (1944), who studied conjugation of phenol by surviving tissue slices, found that glucuronic acid reversed the inhibition of this process produced by iodoacetate. They also found that feeding phenol and borneol to rats increased phenol conjugation by liver and kidney *in vitro*. Their results are difficult to interpret since it is known that phenol may be conjugated with either sulphuric or glucuronic acid. Even if De Meio & Arnolt are correct in thinking that, contrary to the views of Lipschitz & Bueding (1939), glucuronides are formed by direct condensation of the 'aglucurone' with free glucuronic acid, there is no reason to believe that β-glucuronidase is responsible. With regard to De Meio & Arnolt's second finding, there is, in view of our own work, no need to postulate adaptation by the enzyme or enzymes responsible for conjugation of phenol, since both phenol and borneol may have caused liver and kidney damage in their experiments. Results obtained by Bueding & Ladewig (1939) in studying the effect of chloroform poisoning in guinea pigs on glucuronide synthesis by liver slices are of interest in this connexion. Not only did the liver slices from the poisoned animals show the usual increase in glucuronide synthesis on addition of lactate, but they were apparently more active in forming borneol glucuronide than slices from normal animals. The latter aspect of their results is not touched upon by the authors. While there is thus some evidence to suggest that, following damage to an organ, there may be an increase in its ability to form conjugated glucuronides, it is at present impossible to say whether or not this is due to the rise in β-glucuronidase activity observed when repair is in progress, nor is it certain that the glucuronides are formed directly from free glucuronic acid.

SUMMARY

1. The effect of menthol administration to mice in increasing the β-glucuronidase activity of liver and kidney is due to its toxic action on these organs. The rise in enzyme activity is associated with an increase in cell proliferation following injury. Menthol had no effect on spleen glucuronidase.

2. Among other substances examined, the following caused changes in glucuronidase in liver or kidney in an analogous fashion to menthol: chloroform, carbon tetrachloride, mercuric nitrate, yellow phosphorus, phenylarsenoxide, uranyl acetate, menthol glucuronide and sulphathiazole. The effect of chloroform on kidney glucuronidase was confined to male mice.

3. Livers from adult mice after sub-total hepatectomy, and spleens, livers and kidneys from infant mice showed high glucuronidase activities.

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