SUMMARY

1. The component of crude mushroom extracts which is responsible for the appearance of a purple colour when such extracts oxidize catechol has been identified by isolation of L-proline.

2. The pigment-forming reaction has been studied in respect of colour intensity developed, oxygen absorbed and hydrogen ion liberated, in systems containing purified tyrosinase, catechol or homocatechol, and one of the following: proline, hydroxyproline, hydroxyproline ethyl ester, pyrrolidine, glycine, dimethylamine and methylamine. A few experiments were also carried out with alanine, glutamic acid and arginine. The same intense purple colour was obtained in each case in which the nitrogenous substance present had a secondary amino group; the compounds with primary amino nitrogen gave a much less intense orange-red colour.

3. From homocatechol and hydroxyproline ethyl ester, by oxidation with silver oxide in ethanol, 4-(4′-hydroxy-2′-carbethoxy-1′-pyrrolidyl)-5-methyl-*o*-benzoquinone has been prepared in a pure state. It appears to be identical with the pigment formed from the same precursors in the enzymic reaction mixture, and it is concluded that all the pigments have an analogous structure, and that the pigment-forming reaction is essentially a condensation between 1 mol. of *o*-quinone and 1 mol. of nitrogenous base to give a leuco pigment which is spontaneously oxidized to the coloured form by oxygen.

4. In the case of glycine, when this is present in excess, the formation of pigment is followed by oxidation of the excess amino-acid, with liberation of ammonia. Further oxidation of this type does not take place in the case of the imino acid pigments.

REFERENCES

Chodat, R. & Schweizer, K. (1913). *Arch. Sci. phys. nat.* **35**, 140.
Dawson, C. R. & Nelson, J. M. (1938). *J. Amer. chem. Soc.* **60**, 250.
Fischer, E. & Schrader, H. (1910). *Ber. dtsch. chem. Ges.* **43**, 525.
Fischer, E. & Zemplen, G. (1909). *Ber. dtsch. chem. Ges.* **42**, 2989.
Happold, F. C. & Raper, H. S. (1925). *Biochem. J.* **19**, 92.
Jackson, H. (1939). *Biochem. J.* **33**, 1452.
Jackson, H. & Kendal, L. P. (1940). *J. Soc. chem. Ind., Lond.*, **18**, 850.
Kapfhammer, J. & Eck, R. (1927). *Hoppe-Seyl. Z.* **170**, 294.
Keilin, D. & Mann, T. (1938). *Proc. Roy. Soc.* B, **125**, 187.
Kossel, A. & Dakin, H. D. (1904). *Hoppe-Seyl. Z.* **41**, 407.
Platt, B. S. & Wormall, A. (1927). *Biochem. J.* **21**, 26.
Pugh, C. E. M. & Raper, H. S. (1927). *Biochem. J.* **21**, 1370.
Suchanek, O. (1914). *J. prakt. Chem.* **90**, 467.
Szent-Györgyi, A. (1925). *Biochem. Z.* **162**, 399.
Wagreich, H. & Nelson, J. M. (1938). *J. Amer. chem. Soc.* **60**, 1545.
Westerfield, W. W. & Lowe, C. (1942). *J. biol. Chem.* **145**, 463.

β-Glucuronidase as an Index of Growth in the Uterus and other Organs

By LYNDA M. H. KERR, J. G. CAMPBELL and G. A. LEVVY (Imperial Chemical Industries Research Fellow), *Department of Biochemistry and Poultry Research Centre, University of Edinburgh*

(*Received* 5 *November* 1948)

The β-glucuronidase activity of mouse liver or kidney has been shown to be related to the degree of cell proliferation in progress (Levvy, Kerr & Campbell, 1948). It was suggested that the rise in uterine glucuronidase observed after administration of oestrogens to ovariectomized mice (Fishman & Fishman, 1944; Fishman, 1947) could also be explained by cell proliferation.

A comparative study has been made of the kinetics of hydrolysis of phenylglucuronide by β-glucuronidase from mouse uterus, liver and kidney, and of the effects on the enzyme activities of various measures designed to produce proliferative changes in one or more of these organs. Mills (1947) showed that ox-spleen glucuronidase could be separated into two fractions, *A* and *B*, with slightly different pH optima for the hydrolysis of menthylglucuronide. Both these fractions have been found in mouse liver and kidney, while uterine glucuronidase appears to be composed entirely of *A*. No evidence has, however, been obtained to suggest that the effect of an extrinsic agent on the glucuronidase activity of an organ is dependent upon which fraction happens to be present. As in liver and kidney, changes in the enzyme level in uterus resulting from a variety of causes appear to be associated with alterations in growth.

In the course of these experiments, some unexpected changes in glucuronidase activity were encountered. In ovariectomized mice, measures

designed to cause a rise in glucuronidase in liver also produced an increase in uterus, whilst an elevated enzyme activity in liver as well as in uterus was seen after administration of oestrone. These effects have been further investigated.

A preliminary account of part of this work has been published elsewhere (Kerr & Levvy, 1948).

EXPERIMENTAL

Enzyme assay. To permit determination of β-glucuronidase activity in a single mouse uterus, the procedure previously described (Kerr, Graham & Levvy, 1948) was adapted for use with the microcells of the Spekker absorptiometer. As before, the tissue homogenate was freed from inactive protein by maintaining it at pH 5·2 and 38° for 30 min., and the enzyme was precipitated by addition of an equal volume of saturated $(NH_4)_2SO_4$ solution. The enzyme was dissolved in a volume of water such that 0·2 ml. of the resulting solution gave a final reading of 2–4 μg. phenol after correction for blanks. This volume of the enzyme solution was added to 0·1 ml. 0·06 M-phenylglucuronide and 0·1 ml. 0·1 M-citrate buffer at the appropriate pH (see below). After incubation of the hydrolysis mixture for 1 hr. at 38°, 0·5 ml. of a 1 in 5 dilution of Folin-Ciocalteu reagent was added. Protein was removed by centrifuging, and 0·5 ml. of supernatant transferred to a tube containing 0·5 ml. 1·33 N-Na_2CO_3. Colour development was carried out for 20 min. at 38°, and the results were read from a graph constructed with standard phenol solutions put through the same procedure. Assays were done in duplicate, and enzyme and substrate controls were performed as usual. This technique was also adopted for determinations of liver and kidney glucuronidase in the experiments described below, and results are shown in terms of glucuronidase units (G.U.)/g. moist tissue, where 1 G.U. liberates 1 μg. phenol under the standard conditions.

Weight of uterus. Before determining the moist weight of uterus, the tissue was freed from intrauterine fluid by pressing it between pieces of filter paper. The figure then obtained was found to bear a constant relation to the weight after drying at 110° for all conditions of the uterus. No error was introduced into the enzyme assay since the intrauterine fluid contained no detectable amounts of glucuronidase.

RESULTS

Kinetic studies. The pH-activity curve for hydrolysis of phenylglucuronide by mouse-kidney glucuronidase resembled those previously obtained for liver and spleen (Kerr *et al.* 1948) in having two peaks, one at pH 4·5 and the other at pH 5·2. In the case of uterus, however, the activity curve was symmetrical about pH 4·5, and this was still true when the initial purification of the homogenate was omitted. Changes in the enzyme activity in liver, kidney or uterus were not associated with any alteration in the shape of the pH-activity curve (Figs. 1–3). To cover the pH range it was necessary in the case of uterus to pool preparations from two or more mice. For liver and kidney this was only necessary with infant mice. The high figure for uterine glucuronidase in infant mice supports the view that in this organ, as in others, the activity of the enzyme is a measure of growth processes.

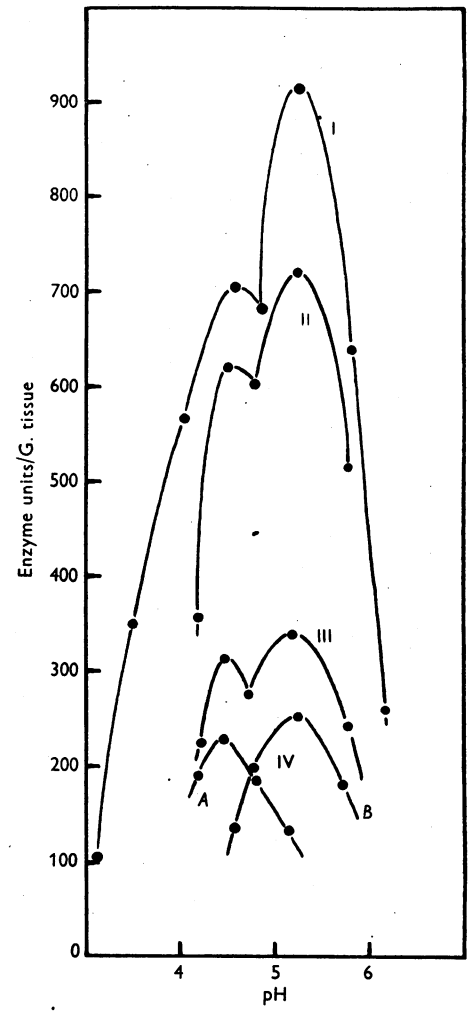

Fig. 1. pH-Activity curves for liver glucuronidase. I, 6-day-old mice; II, adult, 1 day after subcutaneous injection of 5 g. CCl_4/kg.; III, normal adult; IV, the same preparation as III after separation of fractions *A* and *B*.

It was considered that the shapes of the curves for the hydrolysis of phenylglucuronide by mouse liver or kidney indicated the presence of the two glucuronidase fractions found by Mills (1947) in ox spleen, and his technique was applied to their separation. After the preliminary removal of inactive protein by incubation for 30 min. at pH 5·2, the homogenate was made 31·5% saturated with ammonium sulphate. The precipitate thus obtained was devoid of glucuronidase activity. On bringing the preparation to 38·5% saturation with ammonium sulphate, a

large part of the enzyme was precipitated (fraction *A*), whilst all residual activity was removed from solution when the ammonium sulphate concentration was increased to 44·0% saturation (fraction *B*). The separation of the two peaks in the pH-activity curves for liver and kidney achieved in this way is illustrated in Figs. 1 and 2, and it appears that the shapes of the original curves can in fact be explained in terms of Mills's (1947) two fractions. In the fractionation of uterine preparations, all enzyme activity was found in fraction *A*.

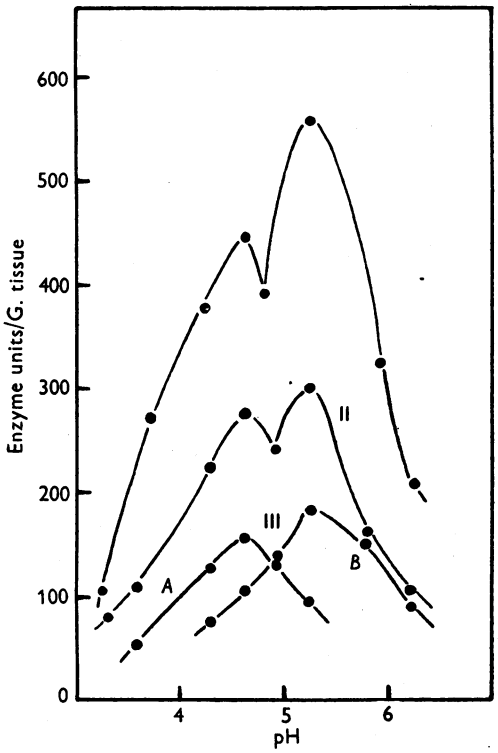

Fig. 2. pH-Activity curves for kidney glucuronidase. I, 6-day-old mice; II, normal adult; III, the same preparation as II after separation of fractions *A* and *B*.

The effect of varying the substrate concentration was studied with uterine enzyme and with fractions *A* and *B* from liver. In every case the activity curve closely resembled that obtained with liver before separation of the two fractions (Kerr *et al.* 1948). K_m, the substrate concentration at which half the observed maximum velocity of hydrolysis was attained, was approximately the same for the two glucuronidase fractions at the figure for the total enzyme in liver (0·0035M).

Mills (1948) has recently published figures for the pH optima in the hydrolysis of phenylglucuronide by his two glucuronidase fractions from ox spleen, and these correspond exactly with the peaks in the pH-activity curves for mouse liver, kidney and spleen.

The effects of various agents on fractions A and B in liver and kidney

The possibility was considered that differences between glucuronidase fractions *A* and *B* in their distribution and response to extrinsic agents might explain the selective actions of such agents on various organs. The nature of the effect of carbon

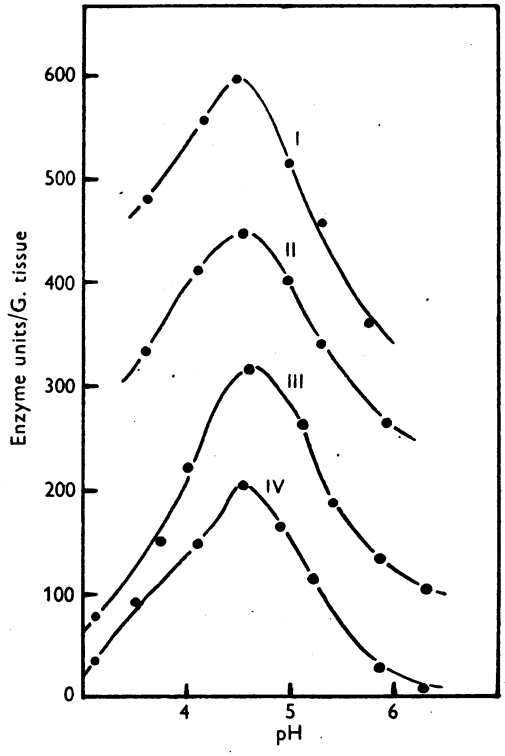

Fig. 3. pH-Activity curves for uterine glucuronidase. I, 10-day-old mice; II, ovariectomized adults, 3 days after subcutaneous injection of 1·7 mg. oestrone/kg.; III, normal adults; IV, ovariectomized adults.

tetrachloride on the pH-activity curve for liver (Fig. 1) renders this possibility unlikely, since both fractions were equally affected in the increase in activity. Carbon tetrachloride is known to be without effect on kidney glucuronidase (Levvy *et al.* 1948), although this organ resembles liver in the composition of the enzyme. In spite of these findings a great many more experiments were done before the possibility just outlined was rejected. In these experiments, the homogenate from each organ was divided into two portions, in one of which *A* and *B* were separated as described above and determined at their respective pH optima, 4·5 and 5·2. The other

portion of the homogenate was brought to 50% saturation with ammonium sulphate and the total enzyme thus precipitated was determined at pH 5·2. Average results for liver and kidney under a variety of conditions are shown in Table 1. Since *A* was determined at a different pH from the total activity, figures for the latter do not agree with the sums of the two fractions. One point not brought out in the table is that individual mice, treated and untreated, showed considerable variation in both liver and kidney in the ratio of the two fractions. Very occasionally, one animal in a group displayed complete lack of *A* or *B* in one of the two organs examined, unaccompanied by any compensatory increase in the activity of the remaining fraction. When this occurred, the size of the group was reduced by one in calculating the average and standard error for the fraction in question, as shown in Table 1.

Table 1. *Effects of various agents on glucuronidase fractions A and B in liver and kidney*

(All values are given as mean ±S.E., followed (in parentheses) by the number of animals in the group.)

Agent	Sex*	Days after treatment	G.U./g. moist tissue: Liver *A*†	Liver *B*‡	Liver Total‡	Kidney *A*†	Kidney *B*‡	Kidney Total‡
None	M.	—	106±17 (6)	223±12 (6)	281±20 (6)	124±16 (6)	123±38 (6)	266±31 (6)
	F.	—	115±12 (6)	247±38 (6)	334±48 (6)	109±8 (6)	118±16 (6)	266±39 (6)
	cM.	—	127±13 (6)	217±18 (6)	301±18 (6)	130±22 (6)	194±18 (6)	286±20 (6)
	cF.	—	116±12 (6)	167±47 (6)	250±49 (6)	113±10 (6)	130±22 (6)	261±43 (6)
Carbon tetrachloride (5·3 g./kg.)	M.	1	545±18 (3)	654±40 (2)§	830±62 (3)	122±27 (3)	150±61 (3)	206±28 (3)
	M.	4	364±58 (3)	537±27 (3)	763±65 (3)	118±19 (3)	138±18 (3)	257±19 (3)
	cF.	4	499±109 (3)	588±24 (3)	664±57 (3)	126±14 (3)	158±6 (3)	235±23 (3)
	cF.	7	517±27 (3)	568±35 (3)	693±31 (3)	127±14 (3)	186±70 (3)	270±42 (3)
Mercuric nitrate (20 mg./kg.)	M.	3	—	—	—	269±35 (3)	319±55 (3)	463±59 (3)
	M.	6	—	—	—	122±14 (3)	158±15 (2)§	277±19 (3)
	F.	3	—	—	—	224±24 (3)	351±45 (3)	491±62 (3)
	F.	6	—	—	—	122±10 (3)	124±26 (3)	237±61 (3)
Chloroform (2 g./kg.)	M.	1	343±188 (6)	766±147 (6)	881±163 (6)	134±19 (6)	121±17 (6)	206±19 (6)
	M.	4	260±42 (3)	561±44 (3)	766±46 (3)	128±18 (3)	234±22 (3)	302±12 (3)
	M.	7	184±29 (6)	559±38 (6)	650±72 (6)	132±29 (6)	395±54 (6)	524±38 (6)
	F.	1	537±64 (6)	410±194 (6)	812±91 (6)	173±10 (6)	135±9 (6)	227±23 (6)
	F.	4	271±45 (3)	570±52 (3)	696±61 (3)	126±19 (3)	148±35 (3)	238±27 (3)
	F.	7	151±42 (6)	559±106 (6)	705±64 (6)	108±16 (6)	223±78 (6)	310±55 (6)
	cM.	1	453±39 (3)	537±25 (3)	659±174 (3)	119±12 (3)	141±17 (3)	221±38 (3)
	cM.	7	152±20 (3)	549±42 (3)	673±32 (3)	132±20 (3)	124±22 (3)	238±30 (3)
	cF.	1	277±22 (2)§	594±32 (2)§	515±23 (3)	187±21 (6)	256±29 (6)	398±67 (6)
	cF.	4	224±25 (3)	510±20 (3)	620±26 (3)	189±15 (3)	247±18 (3)	352±24 (3)
	cF.	7	359±130 (5)	569±39 (5)	657±78 (5)	175±32 (7)	260±69 (7)	359±67 (7)

* c = castrated.
† One G.U. (glucuronidase unit) liberates 1 μg. phenol from 0·015 M-phenylglucuronide in 1 hr. at 38° and pH 4·5.
‡ One G.U. (glucuronidase unit) liberates 1 μg. phenol from 0·015 M-phenylglucuronide in 1 hr. at 38° and pH 5·2.
§ One animal in group devoid of this fraction.

From Table 1, it appears that in either liver or kidney the distribution of glucuronidase activity between the two fractions was on the average the same for normal male and female mice. Castration 3–4 weeks before sacrifice had no marked effect on the results in either sex.

Results obtained during the prolonged rise in liver glucuronidase activity which follows subcutaneous injection of carbon tetrachloride in olive oil were in entire agreement with the conclusions arrived at above, in that this agent showed no discrimination between the two fractions in liver, and was without effect on either in kidney. After subcutaneous injection of mercuric nitrate as an aqueous solution, *A* and *B* rose and fell together in kidney as repair processes became active and were completed. Since mercuric nitrate has little effect on liver (see Table 3), its action on this organ was not studied in the present experiments.

Changes in liver glucuronidase after subcutaneous injection of chloroform in olive oil closely resembled, as one might expect from previous work, those produced by carbon tetrachloride. Taking the results as a whole, there was a suggestion that fraction *A* returned to normal more rapidly than *B*. Before dealing with the effects of chloroform on kidney glucuronidase, it is necessary to consider the influence of sex on the susceptibility of the mouse kidney to chloroform necrosis. Eschenbrenner (1944) found that chloroform caused renal necrosis in male, but not in female mice, and it was shown later (Eschenbrenner & Miller, 1945) that the effect in males was prevented by castration at an early age. The sex-linked nature of this response was associated with

differences in the structure of Bowman's capsule described by Crabtree (1941), but chloroform necrosis, which involved the convoluted tubules, did not extend to the capsule itself. In previous work on glucuronidase (Levvy *et al.* 1948), the effect of chloroform on the kidney-enzyme level in normal mice was seen to be confined to males. From the figures for total kidney glucuronidase in Table 1, it appears that the response in the male was abolished by castration 3 weeks previously, although this operation was not performed until the animals were adult. There was no necrosis, and Bowman's capsule had become predominantly female in character. In ovariectomized females, chloroform caused a small, but significant rise in the kidney enzyme, associated with necrosis and repair (for results one day after injection, $P = 0{\cdot}02$; grouping results for all three time intervals, $P < 0{\cdot}01$). Extending the period between ovariectomy and injection of chloroform from 3 to 13 weeks did not appreciably affect this response, but in the interval a change towards the male type of kidney became much more pronounced. As already noted, figures for uninjected mice showed no variations in *A* or *B*, corresponding to the changes in kidney morphology. The fact that in the male kidney the rise in glucuronidase appeared to be confined to fraction *B* may reflect an uneven distribution of the two fractions throughout this organ, with predominance of *B* in the convoluted tubules. In a histochemical study, Friedenwald & Becker (1948) found greater glucuronidase activity in rat-kidney tubules than in the glomeruli, when hydrolysis of suitable glucuronides in unfixed, frozen sections was allowed to proceed at pH 5.

The effects of sex hormones on liver and uterine glucuronidase

Fishman (1947) has examined the effects of testosterone propionate and oestradiol benzoate, separately and in combination, on uterine glucuronidase in ovariectomized mice. In the doses used, testosterone did not antagonize the action of the oestrogen in causing a rise in the enzyme level, and Fishman interpreted this as indicating 'a unique type of specificity of action by the oestrogen'. His results, however, show that administration of the androgen along with the oestrogen did not entirely prevent an increase in the wet weight of the uterus. By itself, testosterone produced a rise in glucuronidase activity and an increase in weight. Fishman's results seem entirely compatible with the view that an increase in glucuronidase activity in uterus, as elsewhere, reflects increased growth, and that his failure to observe antagonism between oestrogen and androgen resulted from use of too great an excess of the latter. Figures for uterus shown in Table 2 bear out this argument.

Oestrone and testosterone were given as single subcutaneous injections of the solutions in olive oil, alone or within 3 hr. of each other. Four days after injection of ovariectomized mice with 1·7 mg. oestrone/kg., uterine glucuronidase activity and weight were much greater than in untreated controls. The effects of 0·3 mg. oestrone/kg. and 3·3 mg. testosterone/kg. were alike in that there was a comparatively small rise in uterine weight, with a barely perceptible increase in the enzyme activity ($P = 0{\cdot}1$ and 0·02 respectively at the maximum activity). In a smaller dose (2 mg./kg.), testosterone had no action on the uterus, but this dose completely antagonized the effects of the larger dose of oestrone on the enzyme and the weight.

In the experiments with oestrone and testosterone, glucuronidase was determined in liver and kidney as well as in uterus. In ovariectomized mice a marked rise in liver glucuronidase, preceding that in uterus, was observed after injection of oestrone in a dose of 1·7 mg./kg. (Table 2). This effect was also seen in normal and castrate males, but was absent in intact females, even after 4·3 mg. oestrone/kg. Reducing the dose of oestrone to 0·3 mg./kg. abolished the action on liver in ovariectomized mice. Histological examination revealed intense mitotic activity, with little evidence of damage in the livers of oestrone-treated castrate males and females. In normal males, the effect on mitosis was slight, but there was a marked increase in binucleate cells. Testosterone, itself without any action on the liver, antagonized the stimulant effect of oestrone on the enzyme and on cell division. Bullough (1946) has studied the effects of oestrone on mitotic activity throughout the body of the adult female mouse, and concluded 'that those substances which have come to be called oestrogenic or female sex hormones are in fact general mitosis stimulators'. Oestrone produced no effect on liver in his experiments, which were, however, confined to the normal female.

Fractionation of the glucuronidase preparations was carried out in many of the experiments listed in Table 2. Fractions *A* and *B* were both involved in the liver response to oestrone, while all uterine activity was invariably found in fraction *A*.

The effects of liver regeneration on uterine enzyme and weight

Administration of carbon tetrachloride to rats has been shown to produce an increase in the weight of the uterus in immature animals (Talbot, 1939), and to enhance the effectiveness of administered oestrone in ovariectomized animals (Pincus & Martin, 1940). Partial hepatectomy causes a similar increase in the potency of administered oestrogen (Segaloff, 1946). From the work of Roberts & Szego (1947) it appears that increased sensitivity to oestrogens occurs during active liver regeneration rather than in the initial stages of injury. In all these studies the animals were treated with an oestrogen or alternatively the ovaries were still present.

It was, therefore, with surprise that increases in the β-glucuronidase activity and the weight of

Table 2. *Effects of oestrone and testosterone on liver, kidney and uterine glucuronidase*

(All values are given as mean ±S.E., followed (in parentheses) by the number of animals in the group.)

Treatment	Sex*	Days after treatment	Total G.U./g. tissue			Uterine weight (mg.)
			Liver‡	Kidney‡	Uterus†	
None	F.	—	334±48 (6)	266±39 (6)	333±53 (6)	234±56 (6)
	cF.	—	250±49 (6)	261±43 (6)	174±45 (6)	34±18 (6)
	M.	—	281±20 (6)	266±31 (6)	—	—
	cM.	—	301±18 (6)	286±20 (6)	—	—
Oestrone (4·3 mg./kg.)	F.	1	258±39 (3)	370±33 (3)	463±41 (3)	167±66 (3)
	F.	4	244±32 (3)	357±44 (3)	439±63 (3)	192±76 (3)
Oestrone (1·7 mg./kg.)	F.	1	365±60 (3)	223±47 (3)	388±45 (3)	320±24 (3)
	F.	4	267±26 (3)	253±41 (3)	441±87 (3)	253±31 (3)
	cF.	1	431±42 (3)	313±17 (3)	300±54 (3)	26±5 (3)
	cF.	2	481±34 (3)	275±67 (3)	343±47 (3)	47±8 (3)
	cF.	4	569±73 (6)	281±33 (6)	548±106 (6)	211±20 (6)
	cF.	6	399±67 (3)	356±52 (3)	346±41 (3)	52±10 (3)
	cF.	8	315±18 (3)	299±23 (3)	223±16 (3)	46±5 (3)
	M.	1	879±98 (9)	283±95 (9)	—	—
	M.	4	303±12 (6)	221±50 (6)	—	—
	cM.	1	359±43 (3)	324±22 (3)	—	—
	cM.	4	562±58 (3)	333±52 (3)	—	—
Oestrone (0·3 mg./kg.)	cF.	1	271±51 (3)	359±72 (3)	181±13 (3)	51±10 (3)
	cF.	2	274±34 (3)	321±30 (3)	247±38 (3)	47±2 (3)
	cF.	4	269±34 (3)	327±61 (3)	226±34 (3)	102±15 (3)
	cF.	6	256±38 (3)	305±22 (3)	181±36 (3)	63±9 (3)
Testosterone (3·3 mg./kg.)	cM.	1	271±50 (3)	172±14 (3)	—	—
	cM.	4	311±36 (3)	201±23 (3)	—	—
	F.	1	254±32 (3)	297±20 (3)	289±27 (3)	172±54 (3)
	F.	4	272±40 (3)	343±48 (3)	326±19 (3)	193±35 (3)
	cF.	0·5	236±62 (3)	339±38 (3)	199±20 (3)	57±12 (3)
	cF.	1	289±34 (3)	319±56 (3)	260±14 (3)	101±19 (3)
	cF.	2	284±25 (3)	363±46 (3)	183±35 (3)	44±6 (3)
	cF.	4	251±48 (3)	326±46 (3)	189±39 (3)	35±11 (3)
Testosterone (2 mg./kg.)	cF.	1	253±54 (3)	345±16 (3)	156±31 (3)	32±13 (3)
	cF.	4	259±18 (3)	345±25 (3)	154±31 (3)	39±9 (3)
Testosterone (2 mg./kg.) + oestrone (1·7 mg./kg.)	cF.	1	266±51 (6)	379±28 (6)	179±34 (6)	46±12 (6)
	cF.	4	279±20 (5)	340±56 (5)	205±31 (5)	47±7 (5)
	M.	1	261±20 (3)	314±31 (3)	—	—
	M.	4	295±78 (3)	323±64 (3)	—	—

*, †, ‡, see Table 1.

uterus were obtained 7 days after injection of ovariectomized mice with chloroform or carbon tetrachloride (Table 3). That these changes were not due to a direct action of the toxic agent on the uterus, but were secondary to the effect on liver, was shown by further experiments in which mice were submitted to partial hepatectomy 3 weeks after ovariectomy, with similar results. With all three methods of treatment, the rise in uterine weight at its greatest was statistically significant ($P < 0.001$). Liver repair is far advanced after this period (Levvy *et al.* 1948). In a separate group of six mice, the uteri were examined histologically 9 days after partial hepatectomy. Metoestrus, pro-oestrus and, in one case, full oestrus were observed, as compared with dioestrus in ovariectomized controls. It should be noted that not more than 40 % of the liver was removed in the partial hepatectomies in the present experiments. Results obtained with mercuric nitrate suggest that changes in kidney are without effect on uterine weight and glucuronidase activity.

Fractionation of uterine-glucuronidase preparations from ovariectomized mice treated with chloroform and carbon tetrachloride showed all activity to be present in fraction *A*.

DISCUSSION

The original purpose behind the experiments described above was to decide whether the sites of action of various agents on glucuronidase activity were determined by differences in the properties of the enzyme. No evidence of this was obtained. An increase in β-glucuronidase activity appeared to be governed solely by the ability of the agent to stimulate growth processes in the organ in question. Results for uterus did, however, emphasize the need for a preliminary kinetic study with each new organ

Table 3. *Effect of liver regeneration on uterine enzyme and weight*

(All values are given as mean ±S.E., followed (in parentheses) by the number of animals in the group.)

Treatment	Sex*	Days after treatment	Total G.U./g. tissue: Liver‡	Kidney‡	Uterus†	Uterine weight (mg.)
None	F.	—	334±48 (6)	266±39 (6)	333±53 (6)	234±56 (6)
	cF.	—	250±49 (6)	261±43 (6)	174±45 (6)	34±18 (6)
Chloroform (2 g./kg.)	F.	1	812±91 (6)	227±23 (6)	265±45 (6)	277±44 (6)
	F.	4	696±61 (3)	238±27 (3)	342±34 (3)	372±36 (3)
	F.	7	705±64 (6)	310±55 (6)	321±51 (6)	306±29 (6)
	cF.	1	515±23 (3)	398±67 (6)	181±33 (3)	28±10 (3)
	cF.	4	620±26 (3)	352±24 (3)	162±31 (3)	33±7 (3)
	cF.	7	657±78 (5)	359±67 (7)	469±51 (7)	103±20 (7)
Chloroform§ (6 g./kg.)	cF.	8	605±75 (3)	435±56 (3)	386±61 (3)	99±18 (3)
	cF.	10	501±30 (3)	335±52 (3)	306±22 (3)	65±12 (3)
Carbon tetrachloride (5·3 g./kg.)	cF.	1	712±55 (3)	301±42 (3)	203±48 (3)	25±6 (3)
	cF.	4	664±57 (3)	235±23 (3)	285±52 (3)	18±9 (3)
	cF.	7	715±60 (6)	290±59 (6)	496±64 (6)	77±23 (6)
	cF.	10	379±56 (3)	292±12 (3)	205±20 (3)	42±5 (3)
Partial hepatectomy	cF.	2	572±53 (3)	284±22 (3)	225±67 (3)	75±14 (3)
	cF.	4	532±83 (3)	305±29 (3)	240±42 (3)	64±8 (3)
	cF.	6	625±89 (3)	342±34 (3)	412±31 (3)	123±14 (3)
	cF.	8	535±61 (6)	328±54 (6)	395±43 (6)	115±19 (6)
	cF.	12	424±41 (3)	329±32 (3)	277±29 (3)	53±6 (3)
Mercuric nitrate (20 mg./kg.)	cF.	3	241±32 (3)	523±27 (3)	179±18 (3)	30±4 (3)
	cF.	6	238±26 (3)	323±24 (3)	168±26 (3)	39±6 (3)

*, †, ‡, see Table 1.
§ Divided into three daily doses of 2 g./kg.; timed from first injection.

examined for glucuronidase activity. The choice of pH 5·2 for determining activities in liver or kidney with phenylglucuronide would seem to be justified for most purposes.

Changes in the susceptibility of the mouse kidney to chloroform necrosis were faithfully reflected in the figures for glucuronidase activity. The value of such figures as a biochemical index of growth is illustrated by the discovery of new facts relating to liver and uterus. The action of oestrone on liver in normal and castrate males and in ovariectomized females, and the antagonizing of this action by testosterone are in accordance with the view of Bullough (1946) that the effects of such hormones are more widespread throughout the body than is generally appreciated. The absence of any action by oestrone on liver in normal females suggests some form of control of this organ by the ovary.

The changes observed in uterus during liver regeneration in the ovariectomized mouse can only be explained on the assumption that the body is capable of producing an extra-ovarian growth hormone for uterus in significant amounts. In this case, there is obvious need for care in interpreting certain experiments (Talbot, 1939; Pincus & Martin, 1940; Segaloff, 1946; Roberts & Szego, 1947) in which the action of liver damage and regeneration in enhancing the effectiveness of administered or ovarian oestrogens is claimed to be due to 'depressed inactivation' or 'accelerated activation' of the hormones. In view of the effect of oestrone on the liver, a hitherto unsuspected complication must be looked for in the action of this compound on the uterus of the ovariectomized mouse.

SUMMARY

1. Both β-glucuronidase fractions found by Mills (1947) in ox spleen are present in mouse liver and kidney, whilst the uterus contains only one of these.

2. The two glucuronidase fractions in liver and kidney respond identically to agents causing changes in the enzyme activity.

3. In the uterus, as in other organs, changes in glucuronidase activity reflect changes in growth, and the action of oestrone on the enzyme is antagonized by testosterone.

4. Oestrone produces marked increases in glucuronidase activity and cell division in the liver in ovariectomized mice. This action, which is also seen in normal and castrate males, but not in normal females, is antagonized by testosterone.

5. During liver regeneration following chloroform or carbon tetrachloride poisoning or partial hepatectomy, uterine weight and glucuronidase activity increase in ovariectomized mice in absence of administered oestrogen.

The authors wish to express their gratitude to Prof. G. F. Marrian, F.R.S., for instruction in ovariectomies and for a gift of oestrone, to Organon Laboratories Ltd. for a gift of testosterone, and to Mr D. Love for technical assistance. One of us (J. G. C.) is in receipt of a grant from the British Empire Cancer Campaign.

REFERENCES

Bullough, W. S. (1946). *Philos. Trans.* B, **231**, 453.
Crabtree, C. (1941). *Endocrinology*, **29**, 197.
Eschenbrenner, A. B. (1944). *J. nat. Cancer Inst.* **5**, 251.
Eschenbrenner, A. B. & Miller, E. (1945). *Science*, **102**, 302.
Fishman, W. H. (1947). *J. biol. Chem.* **159**, 7.
Fishman, W. H. & Fishman, L. W. (1944). *J. biol. Chem.* **152**, 487.
Friedenwald, J. S. & Becker, B. (1948). *J. cell. comp. Physiol.* **31**, 303.
Kerr, L. M. H., Graham, A. F. & Levvy, G. A. (1948). *Biochem. J.* **42**, 191.
Kerr, L. M. H. & Levvy, G. A. (1948). *Nature, Lond.*, **162**, 219.
Levvy, G. A., Kerr, L. M. H. & Campbell, J. G. (1948). *Biochem. J.* **42**, 462.
Mills, G. T. (1947). *Nature, Lond.*, **160**, 638.
Mills, G. T. (1948). *Biochem. J.* **43**, 125.
Pincus, G. & Martin, D. W. (1940). *Endocrinology*, **27**, 838.
Roberts, S. & Szego, C. M. (1947). *Endocrinology*, **40**, 73.
Segaloff, A. (1946). *Endocrinology*, **38**, 212.
Talbot, N. (1939). *Endocrinology*, **25**, 601.

Concentration of Lipids in the Brain of Infants and Adults

By A. C. JOHNSON (National Research Council Fellow)
A. R. McNABB (National Research Council Fellow) and R. J. ROSSITER
Department of Biochemistry, University of Western Ontario, London, Canada

(*Received* 15 *November* 1948)

Histologically it has been shown that the fibre tracts of the brain are not fully myelinated at birth, but that myelination is completed later, coincident with the functional development of the central nervous system. Most of the medullated fibres of the nervous system are in the white matter, whereas most of the bodies of the nerve cells are in the grey matter. In order to investigate, therefore, the lipid components of the myelin sheath, the lipid distribution in the grey matter and white matter of the brain of the newborn infant has been compared with that of the adult brain.

The important lipids of the central nervous system are cerebrosides, cholesterol and the phospholipins, lecithin, sphingomyelin and kephalin. In a previous report (Johnson, McNabb & Rossiter, 1948*a*) it was shown that white matter of brain is distinguished from grey matter by a greater concentration of cerebroside, free cholesterol and sphingomyelin. It was suggested that these lipids, rather than lecithin and kephalin, formed the lipid components of 'myelin'. Additional evidence was obtained for this view when it was found that medullated peripheral nerve was relatively rich in cerebroside, cholesterol and sphingomyelin, thus resembling the white matter of brain rather than grey matter (Johnson, McNabb & Rossiter, 1948*b*). It has now been shown that white matter of adult brain, in which myelination is complete, differs from 'white matter' of newborn infant brain in that it contains a higher concentration of these same lipids (cerebroside, cholesterol and sphingomyelin).

METHODS

The brain was removed from each of five infants and five adults as soon as possible after death. The infants, whose ages ranged from 7 months' gestation (premature) to full term, died at birth or shortly afterwards. Samples of both grey matter and 'white' matter were taken from the cerebral hemispheres. The grey matter consisted of a thin shaving from the surface of the cerebrum. As there was practically no visible white matter in the infant, the 'white' sample was taken from the positions where white matter was known to occur in the adult brain.

A sample of tissue (1–3 g.) was rapidly weighed and repeatedly extracted with 50 ml. portions of a 1:1 ethanol-ether mixture as described previously (Johnson *et al.* 1948*a*). Additional samples of both grey matter and white matter were taken for the determination of the wet-weight to dry-weight ratio. A portion of the fresh tissue was added to a tared covered crucible, the crucible and tissue weighed, dried in an oven at 105° for 24 hr., cooled in a desiccator and re-weighed. From these figures, the water content of the tissue was calculated. The concentrations of cerebroside, free cholesterol, total cholesterol, total phospholipin, monoaminophospholipin and lecithin were determined in samples of the extract as previously described (Johnson *et al.* 1948*a*), and from these figures were calculated the concentrations of ester cholesterol, sphingomyelin and kephalin.

RESULTS

The figures for both white and grey matter of adult brain (Table 1) were similar to those obtained previously, when two human brains only were analyzed (Johnson *et al.* 1948*a*), and were considerably greater than those for infant brain, chiefly