puppy but would not appear to be essential since the three puppies reared on evaporated cow's milk thrive satisfactorily without it. Apart from this, however, bitches' colostrum should provide a valuable source of unspecific cholinesterase in high concentration, and it is hoped to investigate some of the problems arising out of the observations here presented.

SUMMARY

1. In newborn humans, rats, rabbits and cats the cholinesterase activity of the serum tended to be near the adult level and to rise soon afterwards to

about twice the initial value. In guinea pigs the activity did not rise.

2. The colostrum of man and of the cat had negligible cholinesterase activity.

3. In puppies the serum cholinesterase rose to 25 times its birth level in 3 days. This increase appears to be due to the very high cholinesterase activity of bitches' colostrum.

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The Glucuronide-synthesizing System in the Mouse and its Relationship to β -Glucuronidase

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The β -glucuronidase activity of mouse liver, kidney or uterus has been shown to reflect the state of proliferation of the tissue (Levy, Kerr & Campbell, 1948; Kerr, Campbell & Levy, 1949a). It would appear that β -glucuronidase is distinct from the enzyme system responsible for the production of conjugated glucuronides in the body. The work of Lipschitz & Bueding (1939) suggests that this process is more complex than a simple condensation of the aglycone with free glucuronic acid, and that it takes place in liver and kidney only. β -Glucuronidase, on the other hand, is present to a greater or less extent in practically all animal tissues that have so far been examined (see, for example, Oshima, 1934). Karunairatnam & Levy (1949) found that glucuronide synthesis by adult mouse-liver slices was not appreciably impaired by saccharic acid in con-

centrations which caused almost complete inhibition of β -glucuronidase.

The ability of various mouse tissues to synthesize glucuronides has been studied and compared with their glucuronidase activity under conditions leading to changes in the latter.

EXPERIMENTAL

Measurement of glucuronide synthesis. The conversion of o-aminophenol to its glucuronide in sulphate-free bicarbonate Ringer solution was followed by the method of Levy & Storey (1949). Except in the case of lung, the tissue was sliced, and an amount corresponding to not less than 10 mg. dry weight was taken for each estimation. If necessary, slices from more than one animal were pooled. Whenever possible, the estimation was done in quadruplicate in order to reduce the variable error in the procedure (see Levy & Storey, 1949). In the case of lung, the intact lobes were used. Boyland & McDonald (1948) have shown that this is permissible for measurements of metabolism in lung from young adult mice.

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Results are expressed in the tables as $\mu\text{g. o-aminophenol conjugated/g. dry weight of tissue in 1 hr.}$ Both male and female mice were studied, but sex did not have any apparent effect on values for glucuronide synthesis, and it is therefore not usually shown in the tables.

Glucuronidase activity. The activity of this enzyme in tissue extracts was determined by the method of Kerr, Graham & Levvy (1948), and results are shown in terms of glucuronidase units (a.u.)/g. moist tissue, where 1 a.u. liberates $1\mu\text{g. phenol in 1 hr. from } 0.015\text{M-phenylglucuronide at } 37^\circ \text{ and pH } 5.2.$ In the case of tissues in which the kinetics of hydrolysis of phenylglucuronide by the enzyme have not yet been studied, it was assumed that optimal conditions for hydrolysis resemble those found for liver, spleen and kidney (Kerr *et al.* 1948; Kerr *et al.* 1949a).

RESULTS

Comparison of glucuronide synthesis and glucuronidase activity in various tissues. Table 1 shows the glucuronide-synthesizing power of liver, kidney, spleen and lung in young and adult mice, and of two

Also shown in Table 1 are figures for the glucuronidase activity of the various tissues, some quoted from earlier work from this Department, and others especially determined for present purposes. The distribution of this enzyme obviously bears no relation to that of the synthetic system. The higher glucuronidase activity of organs from young mice compared with those from normal adults, already

Fig. 1. The development of the glucuronide-synthesizing system, as measured by the conjugation of *o*-aminophenol, in the liver of the growing mouse (own mixed colony).

Table 1. The glucuronide-synthesizing power and β -glucuronidase activity of various mouse tissues

(When the mean is based on values for individual animals the standard error of the mean for the group is also shown. Figures in brackets are numbers of animals used.)

Tissue	Age of animal	<i>o</i> -Aminophenol conjugated ($\mu\text{g./g. dry weight}$)	Glucuronidase activity/g. moist weight (a.u.)
Liver	Adult	570 ± 43 (41)	273 ± 13 (23)*
Kidney	Adult	150 ± 14 (4)	363 ± 24 (11)*
	9 days	30 (4)	—
	5 days	—	793 (4)*
Lung	Adult	Nil (2)	185 ± 19 (3)
	9 days	Nil (3)	316 ± 22 (3)
Spleen	Adult	Nil (3)	636 ± 70 (23)*
	9 days	Nil (4)	—
	5 days	—	3245 (4)*
Sarcoma (Crocker 180)	Adult	Nil (2)	433 ± 44 (6)
Carcinoma (Imperial Cancer Research Fund 2146)	Adult	Nil (2)	751 ± 75 (6)

* Quoted from Levvy *et al.* 1948.

transplantable mouse tumours. In agreement with Lipschitz & Bueding (1939), the synthetic system was found only in liver and kidney, the latter being much the less active of the two tissues. The ability of kidney slices to synthesize glucuronides was considerably less in young mice than in adults. This was also true for liver, and Fig. 1 shows the development of the synthetic system in the liver of the growing mouse.

Fig. 2. The change in glucuronidase activity in the liver of the growing mouse: ■, strain C57; Δ , strain A; $\blacktriangle$, strain CBA; ●, own mixed colony, August 1947; ○, own mixed colony, February 1949.

noted for liver, kidney, spleen and uterus (Levy *et al.* 1948; Kerr *et al.* 1949a), is also seen in lung. The change in liver glucuronidase activity has now been fully studied from birth until maturity, and the results are shown in Fig. 2. This graph was compiled from results collected over a considerable period of time with four different strains of mice. The glucuronidase activity of the young mice at a given age was remarkably constant for the different strains, and average figures for adults of different strains were indistinguishable from each other.

In agreement with the observations of Fishman & Anlyan (1947) on human tissues, both mouse tumours were high in glucuronidase activity when compared with most normal adult tissues.

The role of glucuronidase in glucuronide synthesis. The possibility had to be considered that the activity of the glucuronide-synthesizing system in the liver does not vary with age, but that the hydrolytic activity of β -glucuronidase at any given age determines the net amount of synthesis which can be measured (compare Figs. 1 and 2). It has already been shown (Karunairatnam & Levvy, 1949) that saccharate in a concentration of $10^{-2}M$ causes almost complete inhibition of glucuronidase in liver or kidney extracts, but has no appreciable effect on glucuronide synthesis by adult mouse-liver slices. In many of the experiments summarized in Table 1, additional determinations of glucuronide synthesis were made in presence of $10^{-2}M$ -saccharate. This substance caused no increase in the synthetic power of infant liver or adult kidney, nor did its presence lead to glucuronide synthesis by lung, spleen or tumour slices. Failure of saccharate to penetrate the cell would appear to be excluded, since Campbell & Levvy (1949) have found that it inhibits hydrolysis of glucuronides by mouse kidney in the two histochemical tests of Friedenwald & Becker (1948). It seems, therefore, that the glucuronidase activity of a tissue has no bearing on its ability to synthesize glucuronides.

The effect of various measures on the glucuronide-synthesizing system in vivo. Various measures leading to changes in the state of proliferation of mouse liver were examined for their action on the glucuronide-synthesizing system. The results are shown in Table 2, and it can be seen that in no instance was there a detectable change in the activity of the synthetic system. Under similar conditions, a rise in glucuronidase activity to two or three times the normal value was seen after partial hepatectomy, or injection of menthol, carbon tetrachloride or oestrone (Levy *et al.* 1948; Kerr *et al.* 1949*a*). Colchicine in the dose shown causes no change in the normal glucuronidase activity of liver, but it does prevent the rise in activity which follows such measures as partial hepatectomy. In the smaller dose, sorbic acid behaves like colchicine, but the larger dose (240 mg./kg.) causes a profound depression in liver and kidney glucuronidase activity in normal mice. The effects of colchicine and sorbic acid on glucuronidase activity appear to be related to their actions as inhibitors of mitosis (Kerr, Campbell & Levvy, 1949*b*).

The effect of various compounds on the synthesis of o-aminophenylglucuronide in vitro. Four of the compounds examined for their action on the glucuronide-synthesizing system *in vivo* were tested *in vitro* for their effect on the conversion of o-amino-

phenol (0.00023M) to its glucuronide by surviving liver slices from normal mice (Table 3). The fifth compound listed in Table 2, oestrone, was too sparingly soluble in water to permit its study in the present experiments.

Table 2. *The effect of various measures on the glucuronide-synthesizing system in mouse liver*

(When the mean is based on values for individual animals the standard error of the mean for the group is also shown. Figures in brackets are numbers of animals used.)

Treatment	Dose (g./kg.)	Days after treatment	o-Aminophenol conjugated/g. dry weight (μ g.)
None	—	—	570 $\pm$ 43 (41)
(-)-Menthol, intraperitoneally in olive oil	0.33	1 3 6	560 $\pm$ 40 (3) 640 (2) 650 (2)
Carbon tetrachloride, subcutaneously in olive oil	5.33	1 3 7	560 $\pm$ 170 (3) 640 $\pm$ 200 (3) 640 $\pm$ 150 (3)
Partial hepatectomy	—	3 7 10	670 $\pm$ 60 (6) 650 $\pm$ 50 (6) 610 $\pm$ 80 (6)
Oestrone, subcutaneously in olive oil (ovariectomized mice)	0.0017	4	580 $\pm$ 70 (3)
Colchicine, subcutaneously in aqueous solution	0.0015	1	450 $\pm$ 80 (6)
Sorbic acid, subcutaneously in aqueous solution	0.24 0.16	4 4	440 $\pm$ 70 (12) 540 $\pm$ 120 (6)

Table 3. *The effect of various compounds on the synthesis of o-aminophenylglucuronide by mouse-liver slices*

Compound	Concentration (M)	o-Aminophenol conjugated (μ g./g. dry weight)		Inhibition (%)
		In controls	In presence of compound	
Sorbic acid	0.01	380	50	87
	0.01	970	370	62
	0.005	970	500	49
Colchicine	0.01	380	140	63
	0.01	530	190	64
	0.005	530	270	49
Carbon tetrachloride	0.0015	490	520	-6
	0.0015	660	520	21
(-)-Menthol	0.001	450	120	73

Colchicine, sorbic acid and menthol were added as aqueous solutions (if necessary, after pH adjustment) during the preparation of the bicarbonate Ringer solution. In the case of CCl_4 , the Ringer solution was made saturated with the compound. In all experiments, controls were done with untreated slices from the same animal. Each determination was done in quadruplicate, and the standard error of the mean is thus about 10% (Levy & Storey, 1949).

As can be seen from Table 3, in the presence of colchicine, sorbic acid or menthol there was a drop in the conversion of *o*-aminophenol to its glucuronide which was outside the range of error. Inhibition was almost 50 % with 0.005M-colchicine or sorbic acid, and 73 % with 0.001M-menthol. Carbon tetrachloride had no appreciable effect in the highest concentration possible (0.0015M). None of the four compounds in question interfered in the colour reaction for *o*-aminophenylglucuronide.

DISCUSSION

From the results of the experiments described above, it seems clear that there are at least two distinct enzyme systems in the mouse concerned with the metabolism of the conjugated glucuronides. One of these, β -glucuronidase, is present in practically every tissue, and its action is probably entirely hydrolytic. The activity of this enzyme in a tissue varies with the degree of cell division in progress. The other enzyme system is responsible for the synthesis of glucuronides, and is probably complex. It has so far been found only in liver and, to a smaller extent, kidney. The activity of this enzyme system in liver is not altered by measures causing changes in the state of proliferation of the tissue. The ability of liver or kidney to synthesize glucuronides does, however, vary with the age of the animal. At birth, the activity of the synthetic system is small or nil, and it only reaches its ultimate value when the animals are 4 or 5 weeks old. Taken together, the two enzyme systems may provide a mechanism for regulating the transport, action and excretion of physiologically active, glucuronidogenic compounds, such as oestriol. Alternatively, their function may be to provide free glucuronic acid or a transformation

product for building up into more complex molecules.

Since menthol is known to form a glucuronide in the presence of surviving liver slices (Lipschitz & Bueding, 1939), its depressant action on the synthesis of *o*-aminophenylglucuronide *in vitro* is probably the result of competition with *o*-aminophenol rather than genuine inhibition of the synthetic mechanism. The inhibitory actions of sorbic acid and colchicine on glucuronide synthesis *in vitro* are difficult to interpret at present. The overall synthetic process is known to be adversely affected by other agents, such as cyanide, fluoride and iodoacetate (Lipschitz & Bueding, 1939), azide and sulphate (Dr I. D. E. Storey, private communication), but their mode of action is in most cases still obscure.

SUMMARY

1. The glucuronide-synthesizing system in the mouse was found only in liver and, to a smaller extent, kidney.
2. Measures causing changes in the state of proliferation of liver had no effect on the activity of the glucuronide-synthesizing system.
3. The activity of the synthetic system was almost nil at birth, and only reached its ultimate value when the mice were more than 1 month old.
4. The effects of various compounds on glucuronide synthesis *in vitro* were studied.
5. On the basis of this work, the enzyme system responsible for glucuronide synthesis can be clearly distinguished from β -glucuronidase.

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