soluble enzyme fraction in the water suspension was taken for assay. In Wistar rats, the total glucuronidase activity in infants soon reaches and exceeds the ultimate adult value, and in general infant liver has a greater percentage of the enzyme in a soluble form than adult.

SUMMARY

1. After homogenizing rat liver in isotonic sucrose solution, nearly all the glucuronidase activity was found in the subcellular particles. Homogenizing in water made a large part of the enzyme soluble, and it was brought completely into solution by the surface-active agent, Triton X-100.

2. The activity of soluble and insoluble rat-liver glucuronidase in water suspensions was greatly reduced by a non-dialysable endogenous inhibitor. Inhibition, which was overcome by Triton X-100, was also seen in rat kidney, but was very slight in mouse liver and kidney. Rat-liver enzyme was powerfully inhibited by mouse-liver and kidney preparations.

3. The inhibitor in rat liver was thermostable at neutral pH. It was not completely sedimented on

the high-speed centrifuge, but was completely precipitated by buffering to pH 5.2. Incubation in acetate buffer, pH 5.2, destroyed the inhibitor in adult-, but not infant-liver preparations.

4. Inhibition varied directly with substrate and inhibitor concentration, and the kinetics were analysed. The term 'combined inhibition' is suggested for this effect.

5. Inhibition by heparin and chondroitin sulphate was studied. The effects differed in important respects from those of the endogenous inhibitor.

6. The glucuronidase activity of newborn rat liver and of rat liver regenerating after partial hepatectomy was less than that of normal adult rat liver.

7. Sources of error in the assay of β -glucuronidase are discussed, with particular respect to the effects of the endogenous inhibitor in rat liver.

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The Antibacterial Effects of Substances Structurally Resembling Maleimide

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A series of structurally related compounds, including unsaturated cyclic imides, unsaturated dicarboxylic acids, and derivatives of 1:4-quinones has been investigated by Friedmann, Marrian & Simon-Reuss (1948*a, b*, 1949), and their action in inhibiting mitoses in cultures of chick fibroblasts was found to parallel their reactivity towards

sulphydryl groups. Since quinones are well known to have some antibacterial effect (Cooper, 1913; Cooper & Nicholas, 1927; Oxford, 1942*a, b*; Oxford & Raistrick, 1942; Armstrong, Spinke & Kahnke, 1943; Barber, 1944; Atkins & Ward, 1945; see also Hoffman-Ostenhof, 1947); it seemed of interest to assay the whole group for antibacterial action.

EXPERIMENTAL AND RESULTS

The antibacterial effect was determined against a Gram-positive organism (*Staphylococcus aureus*, National Collection of Type Cultures (N.C.T.C.) 4163) and against a Gram-negative organism (*Escherichia coli*, N.C.T.C. 86) grown in a glucose broth medium at pH 6.8-7.0 containing the stated dilution of the compound under test. Tests were also carried out in the presence of 10% horse serum. The inoculum was one loopful of an 18-24 hr. subculture grown at 37°. The highest dilutions of the substances causing 50% inhibition of growth after 18 hr. at 37° are given in Table 1. For comparison the dilutions which cause a decrease of

50% in the mitoses of chick fibroblasts *in vitro* are also given (figures derived from Friedmann *et al.* 1948 *a, b*, 1949 and unpublished). The figure in parenthesis after the name of a compound facilitates reference to Tables 1-3.

Another series of experiments was undertaken to decide whether the action of some of these compounds was bactericidal or bacteriostatic. Growth was observed over a period of 6 days and, after 3 and 6 days, portions from each dilution tube were removed and transferred to nutrient agar to see if any viable cells remained. The results are given in Table 2.

The effect of altered pH on the antibacterial activity of some of the quinones was also studied. The results are shown in Table 3.

Table 1. *Antibacterial effects of maleimide and related compounds*

(Dilutions of a 1/1000 soln. causing 50% inhibition of growth.)

Number and compound	<i>Staph. aureus</i>		<i>Esch. coli</i>		Chick fibroblasts 50% inhibition of mitoses
	Alone	+ 10% serum	Alone	+ 10% serum	
1 Maleimide	1/720	1/50	1/950	1/240	(20% at 1/2000)
2 Citraconimide	1/15	(a)	1/30	(a)	1/5000
3 <i>N</i> -Ethylmaleimide	1/320	1/60	1/500	1/120	1/3700
4 Chloromaleimide	1/60	(a)	1/120	(a)	00 (b)
5 Succinimide	(a)	—	(a)	—	00
6 <i>N</i> -Ethyl- α -carboxymethylthiosuccinimide (c)	(a)	—	(a)	—	00
7 <i>N</i> -Ethylmaleimide + glutathione	(a)	—	(a)	—	Approx. 1/3700
8 Maleic acid	(a)	—	(a)	—	1/3000-1/2000
9 Citraconic acid	(a)	—	(a)	—	00
10 Fumaric acid	(a)	—	(a)	—	00
11 Mesaconic acid	(a)	(a)	(a)	—	00
12 Chloromaleic acid	(a)	—	(a)	—	00
13 Carboxymethylthiosuccinic acid (d)	(a)	—	(a)	—	1/700
14 <i>S</i> -1:2-Dicarboxyethylcysteine (d)	(a)	—	(a)	—	1/1200
15 <i>S</i> -1:2-Dicarboxyethylglutathione (d)	(a)	—	(a)	—	Approx. 1/200
16 1:4-Naphthaquinone	1/200	1/50	1/60	1/50	Approx. 1/63000
17 1:4-Dihydroxynaphthalene diphosphate	(a)	—	—	—	1/1000000
18 The same + phosphatase	1/60	1/20	—	—	—
19 1:4-Dihydroxynaphthalene mono(hydrogen succinate)	1/300	1/80	1/30	1/30	00
20 1:4-Dihydroxynaphthalene di(hydrogen succinate)	1/120	1/80	1/15	1/15	—
21 <i>S</i> -2(1:4-Naphthaquinonyl) thiolacetic acid	(a)	—	(a)	—	00
22 <i>S</i> -2(1:4-Naphthaquinonyl) glutathione	1/20	(a)	(a)	—	00
23 2-Methyl-1:4-naphthaquinone	1/300	—	1/30	—	00
24 2-Methyl-1:4-dihydroxynaphthalene diphosphate (e)	(a)	—	(a)	—	1/750
25 The same + phosphatase	1/50	1/8	—	—	—
26 2-Methyl-1:4-dihydroxynaphthalene di(hydrogen succinate)	1/90	(a)	1/15	(a)	1/430-1/850
27 The same + phosphatase	1/50	1/20	—	—	—
28 <i>S</i> -3(2-Methyl-1:4-naphthaquinonyl) thiolacetic acid	(a)	—	(a)	—	00
29 <i>S</i> -3(2-Methyl-1:4-naphthaquinonyl) glutathione	(a)	—	(a)	—	1/1000
30 2-Chloronaphthaquinone	1/200	(f)	(f)	(f)	—
31 <i>S</i> -3(2-Chloro-1:4-naphthaquinonyl) glutathione	(a)	—	(a)	—	00

(a) No inhibition at 1/10.

(b) 00, No mitotic inhibition at approx. 1/1700.

(c) The addition product of *N*-ethylmaleimide and thiolacetic acid (Marrian, 1949).

(d) Addition products of maleic acid with thiolacetic acid, cysteine and glutathione respectively (Morgan & Friedmann, 1938).

(e) Dicalcium salt.

(f) Irregular results obtained.

Table 2. *Bactericidal and bacteriostatic action of certain quinone derivatives, treated with alkaline phosphatase*

		Dilutions of a 1/1000 soln. causing inhibition of <i>Staph. aureus</i> after					
		18 hr.	40 hr.	3 days		5 days	
Number and compound (phosphatase-treated)		50 %	50 %	50 %	Complete destruction	50 %	Complete destruction
26	2-Methyl-1:4-dihydroxynaphthalene di(hydrogen succinate)	1/150	1/100	1/90	1/64	1/90	1/64
	The same with 10 % horse serum	1/25	1/25	1/25	—	1/25	—
24	2-Methyl-1:4-dihydroxynaphthalene diphosphate (dicalcium salt)	1/50	1/25	1/25	1/8	1/12	1/8
	The same with 10 % horse serum and filtered	1/9	1/7	1/7	1/4	1/7	1/4
17	1:4-Dihydroxynaphthalene diphosphate	1/64	1/25	1/25	1/4	1/12	1/4?
	The same with 10 % horse serum and filtered	1/12	1/6	1/6	1/2	1/6	1/2

Table 3. *Effect of pH variation on the antibacterial activity of some quinone derivatives*

		Dilutions of a 1/1000 soln. showing 50 % inhibition of <i>Staph. aureus</i>		
Number and compound		pH	18 hr.	4 days
27	2-Methyl-1:4-naphthaquinone di(hydrogen succinate) (phosphatase-treated)	6.0	1/150	1/50
		6.8	1/120	1/24
		7.3	1/120	1/50
25	2-Methyl-1:4-dihydroxynaphthalene diphosphate (dicalcium salt) (phosphatase-treated)	6.0	1/64	1/24
		6.8	1/28	1/12
		7.3	1/8	1/8
18	1:4-Dihydroxynaphthalene diphosphate (phosphatase-treated)	6.0	1/32	1/12
		6.8	1/50	1/24
		7.3	1/32	1/12

DISCUSSION

The compounds tested are best discussed in three groups: the imides, the unsaturated acids and the quinone derivatives.

Imides (1–6). These, the most interesting of the compounds tested, were notable for the comparatively high activity shown by the unsaturated members of the series against the Gram-negative *Esch. coli*, even when the medium contained 10 % of horse serum, a potent source of sulphhydryl material. In view of the known rapidity with which unsaturated cyclic imides add —SH groups (Friedmann *et al.* 1949; Marrian, 1949) across the unsaturated centre to form a saturated sulphur-substituted imide, it must be assumed that immediate fixation of the free imide is likely. In this connexion, the lack of activity shown by succinimide (5) and by the addition products (6, 7) of *N*-ethylmaleimide with thiolacetic acid and with glutathione is interesting. It should also be noted that addition of thiolacetic acid, but not of glutathione, destroys the antimutic activity of *N*-ethylmaleimide and of

maleimide (cf. 3 and 7) (Friedmann, Marrian & Simon-Reuss, 1952).

Compounds related to maleic acid (8–15). These show no antibacterial activity at the dilutions tested although a few of the series were mitotic inhibitors.

Compounds related to the quinones. In cases where antibiotic activity is shown the compounds are more active against Gram-positive organisms than against Gram-negative, showing marked dissimilarity to the imides in this respect, but in general any activity that is shown against the Gram-negative organism does not appear to be antagonized by horse serum. The pre-addition of —SH compounds to both 2-methyl-1:4-naphthaquinone (23) and to the unsubstituted naphthaquinone (16) give compounds (21, 22, 28, 29, 31) devoid of activity. The differences in activity shown by the water-soluble derivatives of the quinones, the diphosphates (17, 24) and the succinates (19, 20, 26) of the corresponding phenolic reduced substances are interesting. The more easily hydrolysed succinates showed approximately the same activity as the free quinone, but the diphosphates, being more stable,

were quite inactive until treated with alkaline phosphatase. Only partial activity is, however, restored. This confirms the earlier work of Atkins & Ward (1945).

Investigation (Table 2) as to whether the action of the quinone derivatives was bacteriostatic or bactericidal showed that, as expected, lower concentrations of the active compounds reduced growth but left bacteria viable, while higher concentrations caused complete destruction of the organism.

As the pH of the cultures varied from 6.0 to 7.3 the quinones tended to become somewhat less active.

There seems to be no obvious correlation in this series between antibiotic and antimutagenic action.

SUMMARY

1. Certain unsaturated cyclic imides have been shown to have strong antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

2. The corresponding unsaturated dicarboxylic acids are inactive in this respect.

3. Earlier conclusions as to the antibiotic activity of quinones have been confirmed, and it has been shown that addition of sulphhydryl compounds to the quinone destroys the activity. Increasing pH somewhat decreases the activity of the quinones.

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The Effect of Mastitis on the Carotenoids, Vitamin A and Phosphorus Compounds of Milk

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Of the various infections to which cattle are exposed, mastitis is one of the most prevalent and is the cause of great economic losses in all branches of milk production and manufacture (Davis & McClellmont, 1939). Observations dating back many years have shown that mastitis is accompanied by definite changes in the chemical composition of milk. Amburger (1912) showed that protein, fat, lactose, ash, chloride and specific gravity were affected by mastitis, the chloride being affected in the opposite way to the lactose. About the same time other investigators showed similar changes in mastitic milk from sheep and goats (Grimmer, 1926). In the present paper studies of the effects of mastitis on phosphatase and on the partition of phosphorus, carotenoids and vitamin A are reported. At the same time results of analysis for other milk consti-

tuents are included, in order to show that the milk from the cows which were studied had its composition changed by the disease in the same way as had been found by earlier workers. Chanda & Owen (1949, 1951) found the changes in the partition of phosphorus produced by thyroxine and thiouracil treatment could be related to simultaneous changes of phosphatase. Accordingly, phosphatase was also studied in the present experiments.

Grimmer (1926) cites the observations of many earlier workers who studied the effects of mastitis caused by a variety of pathogens and of others who studied 'salty milk'. These early observers, among whom were Bögold & Stein (1890), noted that phosphate in the ash of milk became less as the proportion of sodium increased; and Grimmer remarks: 'Die Zusammensetzung der Asche nähert