The Effect of Parathormone on the Uptake of ^{32}P into Adenosine Triphosphate and Bone Salt in Kittens

By R. G. WHITEHEAD AND S. M. WEIDMANN

Biological Research Unit, Dental School and Hospital, University of Leeds, Leeds 1

(Received 29 July 1958)

Dawson, Weidmann & Jones (1957) showed that in adult cats and rabbits one of the major effects of Para-Thor-Mone (Eli Lilly and Co.) (parathormone) is to decrease the uptake of ^{32}P into the subendosteal layer of the diaphyseal shaft. The effect was maintained in nephrectomized animals and it was concluded that parathormone had a direct effect on bone. It appears that parathormone affects particularly areas of bone with a high specific activity.

In young animals which have received ^{32}P the specific activity of the phosphorus is much greater in all areas of shaft bone than with adult animals under the same conditions, the subperiosteal layer showing a higher activity than the subendosteal layer (Weidmann & Rogers, 1958). The highest specific activities for inorganic phosphate in hard tissues were found in the orthophosphate of calcifying cartilage (Whitehead & Weidmann, 1957). The trichloroacetic acid-soluble fraction of cartilage was also examined for organic phosphorus compounds and adenosine triphosphate, which was the only one present in measurable quantities, showed a high specific activity. The presence of this compound is interesting because adenosine triphosphate has been suggested as an important intermediate in the calcifying mechanism (Cartier & Picard, 1951).

The present work was carried out with two objects in view: (a) to determine the effect of parathormone on bones which acquire different activity after administration of ^{32}P , and (b) to investigate the effect of parathormone on cartilage adenosine triphosphate. It was hoped to elucidate further the mechanism of parathormone action and the significance of adenosine triphosphate in endochondral calcification.

METHODS

Owing to the biological variation of specific activities of the phosphorus in the hard tissues in young animals, the experiments were carried out with litter-mate pairs. Previous results had shown that a reasonable similarity exists within litters.

The experimental kittens, aged between 7 and 15 days, were divided into two groups, those receiving three daily injections of the hormone and those given only one dose. The animals were treated with 200 U.S.P. units of Para-Thor-Mone (Eli Lilly and Co.)/day/kg. body wt., injected intraperitoneally. Certain of the control animals were also

injected with a comparable volume of physiological saline. Whilst undergoing this treatment the experimental and control animals were kept together with the mother. Both groups were injected intraperitoneally with $330\text{ }\mu\text{C}$ of carrier-free ^{32}P /kg. body wt. 16 hr. after the final administration of the hormone. The animals were killed 2 hr. later. Blood samples were taken by heart puncture under anaesthesia immediately before death.

Serum. The serum was analysed for inorganic phosphate and ^{32}P content as previously described (Rogers, Weidmann & Jones, 1953).

Long bones. The tibiae, femora and humeri were dissected and cleaned by the usual techniques. Estimations for ^{32}P uptake in the cortical and cancellous bone were carried out as described by Rogers *et al.* (1953). The ^{32}P uptake in the subendosteal and subperiosteal layers of the femur was measured by extraction with 90% (w/v) saturated ammonium sulphate by the wax-protection method (Weidmann, 1956).

Cartilage. The limb cartilages were analysed as recently described (Whitehead & Weidmann, 1957). Since the trichloroacetic acid extract of cartilage contains only adenosine triphosphate and orthophosphate in detectable quantities an adequate separation of these compounds is achieved by one-dimensional paper chromatography with the solvent and conditions described in the above-mentioned paper.

The specific activities have been expressed throughout this paper as the following ratio:

$$\frac{(\text{Counts/min./mg. of P}) \times 10^9}{\text{Administered counts/min./kg. body wt.}}$$

This formula is similar to that used in previous studies (Rogers *et al.* 1953) except that the factor 10^9 is replaced by 10^6 , because in the present work $330\text{ }\mu\text{C}$ /kg. is used as the standard injection instead of $33\text{ }\mu\text{C}$ /kg.

RESULTS

In order to obtain a clear picture of parathormone action, the weights of the kittens which received multiple injections of hormone were measured each day during the experiment. The average increase in weight of control animals over the period of 3 days was 25 g., whereas the experimental group increased by only 12 g. The growth rate of those controls which were injected with saline was the same as the uninjected animals. Hence parathormone acts to some extent as a growth inhibitor, but the animals continued to feed from the mother during the experiment and were apparently as active as the controls. The reduced growth rate is therefore more likely to be due to a general effect of

parathormone on the metabolism of the animals, than a result of their being too ill to feed. The typical effect of parathormone injection in increasing serum calcium is shown in Table 1, in which an increase in all the pairs can be seen.

The effects on serum inorganic phosphate and on the specific activity of the incorporated ^{32}P were also measured and the results are recorded in Tables 2 and 3. Statistical analysis shows that parathormone produces no significant alteration in either of these values in the two groups studied. This is an important finding because it could be argued that the results for bone presented below are

not due to a specific effect of parathormone on hard tissue, but due to its general effect on phosphorus metabolism, consequently impairing the distribution of isotope. The lack of difference between the experimental and control serum specific activities shows, however, that isotope available for bone-salt labelling is unchanged by the treatment.

Effect of parathormone on the uptake of ^{32}P into bone

In order to compare these results with those of adult animals, the effect of parathormone on the uptake ^{32}P was measured both in average samples of

Table 1. *Effect of parathormone on the concentration of total serum calcium in kittens*

Each figure represents the result from one kitten.

Litter no.	Multiple injections		Litter no.	Single injection	
	Experimental (mg./100 ml.)	Control (mg./100 ml.)		Experimental (mg./100 ml.)	Control (mg./100 ml.)
1	26.1	13.8	8	18.6	10.9
2	25.0	13.4	9	19.3	10.9
3	16.4	12.8	10	21.5	11.4
4	14.0	12.8	11	17.3	11.8
5	—	—	12	17.9	11.2
6	—	—			
7	—	—			

Table 2. *Effect of parathormone on the concentration of serum inorganic phosphorus in kittens, measured 2 hr. after injection of ^{32}P*

Litter no.	Multiple injections			Litter no.	Single injection		
	Experimental (mg. of P/100 ml.)	Control (mg. of P/100 ml.)	Effect (%)		Experimental (mg. of P/100 ml.)	Control (mg. of P/100 ml.)	Effect (%)
1	8.9	9.4	-5	8	9.5	9.5	0
2	10.6	11.7	-9	9	10.6	10.6	0
3	9.7	10.3	-6	10	11.0	11.0	0
4	7.7	10.3	-24	11	9.4	9.7	-3
5	9.4	10.6	-11	12	7.8	8.0	-1
6	11.4	10.6	+9				
7	9.7	8.9	+8				
Average			-5*	Average			-1†

* Not considered significant ($P > 0.3$).

† Not considered significant ($P > 0.2$).

Table 3. *Effect of parathormone on the specific activity of serum inorganic phosphate, measured 2 hr. after injection of ^{32}P*

(a) Multiple injections				(b) Single injection			
Litter no.	Specific activity		Effect (%)	Litter no.	Specific activity		Effect (%)
	Experimental	Control			Experimental	Control	
1	220 000	210 000	+5	8	258 000	264 000	-2
2	185 000	190 000	-3	9	217 000	210 000	+3
3	210 000	230 000	-9	10	209 000	211 000	-19
4	286 000	230 000	+20	11	266 000	236 000	+12
5	185 000	200 000	-7	12	259 000	226 000	+15
6	200 000	200 000	0				
7	206 000	200 000	+3				
Average			+1*	Average			+5†

* Not considered significant ($P < 0.5$).

† Not considered significant ($P < 0.2$).

cortical and cancellous bone, and in the subperiosteal and subendosteal layers of femur. The results for cortical and cancellous bone are presented in Tables 4 and 5. In the cortical bone a small but significant diminution in uptake occurs in both groups studied. There is little difference between the effect of single and repeated injections of parathormone. In the cancellous bone, however, repeated injections of the hormone produce a much greater inhibition of uptake of ^{32}P than overnight injection.

These results are in direct contrast with those found in adult cats and rabbits (Dawson *et al.* 1957), where there was no significant effect on these tissues even after repeated injections of parathormone for 9 days. The results are in agreement with those of Engfeldt & Zetterström (1954), who found similar results to ours in young rats.

Table 6 shows the effect produced in the subperiosteal layer of the femur. A single injection of the hormone results in only a small inhibition of isotope incorporation; multiple dosage, however,

Table 4. *Effect of parathormone on the specific activities of phosphorus in the cortical bone of kittens, measured 2 hr. after injection of ^{32}P*

(a) Multiple injections				(b) Single injection			
Litter no.	Specific activity		Effect (%)	Litter no.	Specific activity		Effect (%)
	Experimental	Control			Experimental	Control	
1	2500	3320	-30	8	5210	7250	-28
2	3650	4050	-10	9	4500	7300	-38
3	3080	4640	-30	10	2850	3290	-14
4	3620	4600	-26	11	4330	6090	-29
5	3640	4500	-19	12	4200	4970	-16
6	4100	4100	-9				
7	3550	3650	-2				
Average			-18*	Average			-25*

* Significant at $P < 0.01$.

Table 5. *Effect of parathormone on the specific activities of phosphorus in the cancellous bone of kittens, measured 2 hr. after injection of ^{32}P*

(a) Multiple injections				(b) Single injection			
Litter no.	Specific activity		Effect (%)	Litter no.	Specific activity		Effect (%)
	Experimental	Control			Experimental	Control	
1	3 060	4 320	-30	8	9 520	10 000	-5
2	5 570	6 120	-9	9	5 690	8 260	-32
3	4 390	10 000	-56	10	3 520	15 160	-30
4	6 900	10 000	-31	11	6 210	9 400	-34
5	3 070	10 000	-69	12	4 570	7 140	-36
6	6 200	10 000	-38				
7	3 570	8 300	-57				
Average			-41*	Average			-25†

* Significant at $P < 0.001$.

† Just significant at $P < 0.05$.

Table 6. *Effect of parathormone on the specific activities of phosphorus in the subperiosteal layer of the femur, measured 2 hr. after injection of ^{32}P*

(a) Multiple injections				(b) Single injection			
Litter no.	Specific activity		Effect (%)	Litter no.	Specific activity		Effect (%)
	Experimental	Control			Experimental	Control	
1	—	—	—	8	25 400	29 300	-14
2	—	—	—	9	20 600	29 000	-29
3	4 840	25 000	-80	10	12 300	22 500	-45
4	13 300	25 300	-48	11	18 400	21 100	-13
5	3 490	27 000	-86	12	20 500	23 300	-12
6	5 460	15 000	-63				
7	3 600	27 000	-86				
Average			-73*	Average			-23†

* Significant at $P < 0.001$.

† Just significant at $P < 0.05$.

results in very marked changes. The subperiosteal layer of the femur in normal kittens has a specific activity five times that of a sample from the whole cortex. In the parathormone-treated animals, the incorporation of isotope into this layer is so much reduced that its specific activity is almost identical with that of the whole tissue. Again, this result is in contrast with that found in adult animals, where no significant effect was detected in this area. The results for the subendosteal layers (Table 7) are similar to those found in the subperiosteal layers; thus the specific activity of phosphorus is con-

siderably reduced in both surfaces of the shaft bone. In adult animals the only effect observed was in the subendosteal area.

The results obtained with the control kittens show an upward gradation in specific activity of incorporated ^{32}P in the increasing order: cortical bone, cancellous bone, subperiosteal layer, the uptake being significantly greater in the subperiosteal layer compared with the cancellous bone, and in the cancellous compared with the cortical bone (difference, $P < 0.01$). On comparison with the corresponding effects produced by multiple

Table 7. *Effect of parathormone on the specific activities of phosphorus in the subendosteal layer of the femur, measured 2 hr. after injection of ^{32}P*

(a) Multiple injections				(b) Single injection			
Litter no.	Specific activity		Effect (%)	Litter no.	Specific activity		Effect (%)
	Experimental	Control			Experimental	Control	
1	—	—	—	8	8 440	9 240	-9
2	—	—	—	9	7 190	9 180	-22
3	4 840	10 600	-56	10	4 460	4 610	-4
4	7 700	10 600	-27	11	6 200	7 680	-19
5	5 300	13 300	-60	12	4 200	5 310	-21
6	5 000	7 600	-37				
7	4 050	13 000	-68				
Average			-50*	Average			-15*

* Significant at $P < 0.01$.

Table 8. *Effect of parathormone on the specific activities of the orthophosphate in the epiphyseal cartilage, measured 2 hr. after injection of ^{32}P*

(a) Multiple injections				(b) Single injection			
Litter no.	Specific activity		Effect (%)	Litter no.	Specific activity		Effect (%)
	Experimental	Control			Experimental	Control	
1	21 000	47 000	-55	8	62 500	75 400	-17
2	40 000	56 000	-29	9	58 500	64 600	-9
3	26 000	55 000	-53	10	49 000	51 800	-5
4	45 000	55 000	-18	11	58 000	64 600	-10
5	33 000	72 000	-54	12	65 000	63 000	+4
6	32 000	68 000	-53				
7	33 000	72 000	-54				
Average			-45*	Average			-7†

* Significant at $P < 0.001$.

† Not significant ($P < 0.1$).

Table 9. *Effect of parathormone on the specific activities of adenosine triphosphate, measured 2 hr. after injection of ^{32}P*

(a) Multiple injections				(b) Single injection			
Litter no.	Specific activity		Effect (%)	Litter no.	Specific activity		Effect (%)
	Experimental	Control			Experimental	Control	
1	10 400	21 300	-52	8	14 600	27 600	-47
2	18 200	25 000	-27	9	17 500	36 000	-51
3	11 000	25 200	-56	10	13 200	26 400	-50
4	18 500	25 000	-27	11	15 000	29 000	-48
5	10 800	38 000	-72	12	20 000	29 600	-30
6	13 500	38 000	-64				
7	12 300	38 000	-68				
Average			-52*	Average			-45*

* Significant at $P < 0.001$.

injection of parathormone, it will be seen that the percentage inhibition produced in the uptake of isotope is 73 % in the subperiosteal layer, which is significantly greater than the 41 % produced in the cancellous bone ($P < 0.01$). Similarly, the cancellous bone is more affected than the cortical bone, where only 18 % reduction is found; the difference between the two groups being just significant ($P < 0.05$). Thus in the long bones parathormone has its greatest effect in areas where the highest ^{32}P uptake is found. The previous observation that, in adult animals, the only area affected was the subendosteal layer, which shows a considerably higher uptake of isotope than any other area, agrees with this generalization.

*Effect of parathormone on the uptake
of ^{32}P into cartilage*

The results obtained from the cartilage estimations are recorded in Tables 8 and 9. It is interesting to compare the specific activities of the ^{32}P incorporated into the orthophosphate and into the adenosine triphosphate. Sixteen hours after a single injection of parathormone, the uptake of ^{32}P into the orthophosphate is very little affected; the adenosine triphosphate turnover is, on the other hand, considerably reduced (45 %). Repeated injections of the hormone have no further significant effect on the adenosine triphosphate turnover, the inhibition remaining at about 52 % below the control values. Repeated injections of the hormone, however, bring about a marked reduction in the turnover of the orthophosphate fraction. It would appear that the action of parathormone results in a very rapid reduction of adenosine triphosphate turnover, which is followed more slowly by inhibition of uptake of ^{32}P into the orthophosphate of bone salt.

DISCUSSION

The uptake of labelled ion into hard tissues results mainly from two reactions: ionic exchange between bone and blood minerals and through mechanisms connected with cellular activity. In growing tissue both these reactions occur to a marked extent, but because of the rapid rate of the ionic-exchange reaction compared with the uptake of isotope resulting from cellular activity, it was suggested that in short-term experiments the physico-chemical factors dominate (Neuman & Neuman, 1953). This might seem to render it impossible to establish a relationship between cellular processes and isotope incorporation in short-term experiments. The present results, and those of Dawson *et al.* (1957), show, however, that in this type of experiment treatment with parathormone produces profound effects on the uptake of ^{32}P into

hard tissues, especially in those areas with a very active metabolism.

The results for the long bones confirm the supposition of Dawson *et al.* (1957) that only the more active areas of bone are susceptible to parathormone action. In the adult animal only the subendosteal layer is affected, i.e. the layer which shows the highest uptake of isotope (Weidmann, 1956). Estimation of whole-bone samples in adult animals showed no effect from hormonal treatment, owing to the presence of a large mass of comparatively inactive bone. In young animals the effect is more widely distributed. The masking effect by the inactive bone is still present, as shown by the 18 % reduction occurring in the whole cortical bone compared with the 60–70 % found in the layers of long bones of animals treated by multiple dosage. These results also demonstrate that the maximum effect of parathormone on the uptake of ^{32}P in bone salt is achieved only with multiple dosage of the hormone. This correlates well with findings in adult cats where the reduced activity in the subendosteal layer was demonstrable only after 9 days' treatment (Dawson *et al.* 1957).

The ossifying cartilage provides the most interesting results. Parathormone has a rapid effect on the metabolism of cartilage cells, as shown by the immediate reduction in turnover of adenosine triphosphate. This effect on energy-rich phosphate metabolism is supported by the recent findings of Dawson & Jones (1957), who have demonstrated that parathormone reduces the ability of aged, washed homogenates of liver and kidney to carry out oxidative phosphorylation. There was a reduced formation of organic phosphate from inorganic phosphate when the cyclophorase system was coupled with a hexokinase preparation. The action of parathormone was also associated with steps of the citric acid cycle between citrate and α -oxoglutarate, and between α -oxoglutarate and succinate. It was suggested that parathormone activity could be explained if the production of adenosine triphosphate in cells was decreased.

Laskin & Engel (1956) have emphasized the probable importance of the citric acid cycle in bone metabolism as a result of their studies in parathormone-treated young albino rabbits. In these animals the respiration of bone slices is considerably reduced, and accompanied by a comparable reduction in succinic dehydrogenase activity. Further evidence of the importance of oxidative processes in the production of energy-rich phosphate in cartilage is provided by unpublished results of the present authors. Injections of dinitrophenol in sufficiently small amounts to have no obvious effect on the animals produced a very rapid inhibition of turnover of cartilage adenosine triphosphate. The effects were in many respects

comparable with the parathormone results discussed in this paper. It seems therefore reasonable to assume that oxidative phosphorylation plays an important role in the formation of cartilage adenosine triphosphate, and that its reduced turnover resulting from parathormone treatment occurs from inhibition of this mechanism.

Adenosine triphosphate has for some time been suspected as an intermediate in the calcification mechanism. Cartier & Picard (1951) showed *in vitro* that at a physiological pH adenosine triphosphate produces much more intense calcification than any other phosphate ester. More recently they have demonstrated (Cartier & Picard, 1955*a, b, c*; Cartier, 1957) that enzyme systems utilizing adenosine triphosphate are important only in the initial stages of calcification. According to these authors the fixation of pyrophosphate has an initiating effect on the deposition of tricalcium phosphate. Further evidence of this stimulating effect of adenosine triphosphate on the calcification mechanism comes from the work of Sobel, Burger & Deane (1957), who found that prior treatment with adenosine triphosphate of chondroitin sulphate containing demineralized bone enables recalcification to occur *in vitro* at physiological concentrations of calcium and phosphorus. This evidence suggests that adenosine triphosphate is important in the formation of a primary seed, after which bone salt crystallizes out automatically and independently of cellular processes. If this is the case then any factor affecting adenosine triphosphate production would be expected to have a marked effect on bone-salt formation. The possible role of a seeding mechanism in relation to bone-salt formation has recently been reviewed by Neuman & Neuman (1958). If it is assumed that adenosine triphosphate is an intermediate in the seeding mechanism of hard tissues then the present results can be interpreted in the following way. The uptake of ^{32}P into hard tissue of young animals will depend (*a*) on the formation of the seed by cellular processes, (*b*) on the newly formed bone salt which is automatically crystallized out on to the seed, and (*c*) on the high ionic exchange always associated with newly formed bone salt (Neuman & Neuman, 1957). At present the possibility cannot be excluded that the reduced metabolism of adenosine triphosphate could also result in an impaired matrix formation.

At short time intervals after injection of the parathormone into the animals, although the turnover of the adenosine triphosphate is rapidly reduced, the effect of this is not immediately observed in the incorporation of ^{32}P into bone salt. It is suggested that there is an excess of seed which as it were buffers the inorganic phosphate against changes in adenosine triphosphate metabolism. This assumption

is supported by the fact that cartilage orthophosphate is little affected after overnight treatment with the hormone, whereas the adenosine triphosphate shows a considerably reduced incorporation of the isotope. As time proceeds the reduced metabolism of adenosine triphosphate will result in a diminished seed formation and, as a consequence of this, less crystallization of bone salt will take place, which will result not only in a reduced incorporation of isotope from this source but also from a subsequently reduced ionic exchange. This could explain the delayed effect of parathormone on bone salt.

Results obtained with parathormone require careful interpretation because of the impurity of the preparation. Recently Rasmussen (1957) has prepared a sample of parathyroid extract which moves as a single zone on electrophoresis and as a single peak in the ultracentrifuge. This preparation causes both serum calcium rise and serum phosphate fall. J. Dawson & E. A. Jones (personal communication) have recently used a sample of this pure preparation. It inhibited oxidative phosphorylation in a similar way to Para-Thor-Mone (Eli Lilly and Co.), the preparation used in the present work. This provides evidence for the possibility that the results presented here are a true action of the parathyroid hormone.

SUMMARY

1. Parathormone reduces the uptake of ^{32}P into all the areas of hard tissue studied in young kittens; the effect is greatest in the most active areas.

2. Parathormone causes a reduced incorporation of ^{32}P into cartilage adenosine triphosphate. The possible importance of this ester in the calcification mechanism has been discussed.

3. These effects on hard tissue are accompanied by a reduced growth rate, which suggests a more general effect of parathormone on metabolism.

It is a pleasure to thank Dr J. Dawson for his interest during this work. We would also like to acknowledge the technical assistance given to us by Mrs E. B. Marshall and Mr G. E. A. Moreton. This work has been supported by a grant from the Medical Research Council.

REFERENCES

- Cartier, P. (1957). *Bull. Soc. Chim. biol., Paris*, **39**, 169.
- Cartier, P. & Picard, J. (1951). *C.R. Soc. Biol., Paris*, **145**, 274.
- Cartier, P. & Picard, J. (1955*a*). *Bull. Soc. Chim. biol., Paris*, **37**, 661.
- Cartier, P. & Picard, J. (1955*b*). *Bull. Soc. Chim. biol., Paris*, **37**, 1159.

- Cartier, P. & Picard, J. (1955c). *Bull. Soc. Chim. biol., Paris*, **37**, 1169.
- Dawson, J. & Jones, E. A. (1957). *Int. Congr. clin. Chem., Stockholm*, p. 86.
- Dawson, J., Weidmann, S. M. & Jones, H. G. (1957). *Biochem. J.* **66**, 116.
- Engfeldt, B. & Zetterström, R. (1954). *Endocrinology*, **54**, 506.
- Laskin, D. M. & Engel, M. B. (1956). *Arch. Path.* **62**, 296.
- Neuman, W. F. & Neuman, M. W. (1953). *Chem. Rev.* **53**, 1.
- Neuman, W. F. & Neuman, M. W. (1957). *Amer. J. Med.* **22**, 133.
- Neuman, W. F. & Neuman, M. W. (1958). In *The Chemical Dynamics of Bone Mineral*, ch. 7. Chicago: University Press.
- Rasmussen, H. (1957). *J. biol. Chem.* **229**, 781.
- Rogers, H. J., Weidmann, S. M. & Jones, H. G. (1953). *Biochem. J.* **54**, 37.
- Sobel, A. E., Burger, M. & Deane, B. C. (1957). *Int. Congr. clin. Chem., Stockholm*, p. 129.
- Weidmann, S. M. (1956). *Biochem. J.* **62**, 593.
- Weidmann, S. M. & Rogers, H. J. (1958). *Biochem. J.* **69**, 338.
- Whitehead, R. G. & Weidmann, S. M. (1957). *Nature, Lond.*, **180**, 1196.

Mammalian Glycosidases

DISTRIBUTION IN THE BODY

By J. CONCHIE, JUNE FINDLAY AND G. A. LEVY

Rowett Research Institute, Bucksburn, Aberdeenshire

(Received 6 August 1958)

The enzyme β -glucuronidase is universally distributed in mammalian tissues, and has been extensively studied from many aspects (see Levy, 1956; Levy & Marsh, 1959). Investigations on the changes in activity of the enzyme *in vivo* in response to extrinsic agents have been complicated by genetic factors which can lead to considerable variations in activity and response within a species. It has been concluded, however, that the enzyme is under endocrine control, though its function in the body remains obscure.

It was considered that the mammalian cell might contain other glycosidases behaving similarly to β -glucuronidase, and to obtain some preliminary information on this point various tissues have been screened for glycosidase activity with a number of chromogenic substrates. With those enzymes which appeared most active in all the tissues examined, namely α -mannosidase, β -galactosidase and β -N-acetylglucosaminidase, a study has been undertaken of their distribution in the mouse and the rat. For purposes of comparison, β -glucuronidase activities have also been measured, and extended to a number of hitherto unexamined tissues.

Parts of this work have been described in preliminary communications (Conchie, Findlay & Levy, 1956, 1957; Conchie, 1957). Comparative studies of β -glucuronidase, α -mannosidase and β -N-acetylglucosaminidase in cancer tissues have already been published (Conchie & Levy, 1957a), as has the distribution of glycosidases in semen, sperm and male accessory secretions of many mammalian species (Conchie & Mann, 1957).

A study of the changes *in vivo* of α -mannosidase, β -galactosidase, β -N-acetylglucosaminidase and

β -glucuronidase in response to hormones and other extrinsic agents has been made by Conchie & Findlay (1958).

EXPERIMENTAL

Materials

Phenolphthalein β -D-glucuronide was prepared biosynthetically by the method of Talalay, Fishman & Huggins (1946).

o-Nitrophenyl β -D-glucoside, prepared as described for the galactoside (Seidman & Link, 1950), had m.p. 152°, $[\alpha]_D^{16} - 107^\circ$ in water (c, 1.2); Montgomery, Richtmyer & Hudson (1942) give the same constants.

Phenyl α -D-galactoside, prepared as described for the glucoside (Montgomery *et al.* 1942), had m.p. 145°, $[\alpha]_D^{16} + 215^\circ$ in water (c, 1.4); Helferich & Appel (1932) give m.p. 146°, $[\alpha]_D + 217^\circ$.

p-Nitrophenyl α -D-mannoside, prepared as by Jermyn (1955), had m.p. 180–181° and $[\alpha]_D^{19} + 145^\circ$ in water (c, 0.2).

The following compounds were also prepared: *p*-nitrophenyl *N*-acetyl- β -D-glucosaminide (Findlay, Levy & Marsh, 1958); phenyl *N*-acetyl- α -D-glucosaminide and phenyl *N*-acetyl- β -D-glucosaminide (Conchie & Levy, 1957b); *o*-nitrophenyl β -D-galactoside (Seidman & Link, 1950); *p*-nitrophenyl α -D-glucoside and phenyl β -D-xyloside (Montgomery *et al.* 1942); phenyl β -D-mannoside (Helferich & Winkler, 1933); D-mannono-1:4-lactone (Nelson & Cretcher, 1930).

2-Acetamido-2-deoxy-D-gluconolactone (*N*-acetylglucosaminolactone) was prepared by oxidation of *N*-acetylglucosamine with HgO as described by Findlay *et al.* (1958).

D-Galactono-1:4-lactone was obtained from L. Light and Co. Ltd.

Saccharate solutions, prepared from potassium hydrogen saccharate (British Drug Houses Ltd.), were boiled before use for 30 min. to cause one-third conversion into saccharo-1:4-lactone (Levy, 1952).