cornea with vascularization, positive aqueous ray and plastic iritis. The ocular inflammation continued for at least 7 days in the eyes not removed for histological study before this time. The immune but insensitive animals reacted like the normal controls with a purely traumatic reaction that subsided in less than 7 days.

Summary. Rabbits cannot be sensitized to lens protein by ordinary methods. By the intermediary action of Staphylococcus toxin a skin and ocular sensitivity to lens substance has been developed. Precipitin titers, higher than those developed by ordinary methods, are also produced. This method, to our knowledge, has not been previously reported and may be of wider application in the experimental study of hypersensitive states.

A more complete report will be published in an ophthalmological journal.

7164 C

Phosphatase Activity of the Bones and Kidneys in Thyrotoxicosis.*

MERRITT B. LOW, ROBERT O. WILSON AND JOSEPH C. AUB

From the Laboratories of the Collis P. Huntington Memorial Hospital, Harvard University.

Considerable experimental work has been done within the past few years on the phosphatase activity of various bodily tissues. Robison[1] showed that soluble salts of hexosemonophosphoric esters were hydrolyzed by an enzyme present in ossifying cartilage, kidney, and the shafts of long bones. Kay,[2] Robison,[1] and others have shown that the intestinal mucosa, kidney, and whole bone contain the greatest concentration of this enzyme. Furthermore, the plasma and tissue phosphatase activity has been shown to vary from the normal in certain pathological conditions. Page[3] has shown that bone phosphatase is diminished if an excess of vitamin D is fed to an animal, along with the coincident decalcification of bone and metastatic calcification of other tissues. Parathormone has also been shown[4] to

* This is reprint No. 324 of the Cancer Commission of Harvard University.

[1] Robison, R., *Biochem. J.*, 1923, **17**, 286.

[2] Kay, H. D., *Biochem. J.*, 1928, **22**, 855.

[3] Baumgartner, L., King, E. J., and Page, I. H., *Biochem. Z.*, 1929, **213**, 170.

[4] Page, I. H., and Reside, M., *Biochem. Z.*, 1930, **223**, 171; 1930, **226**, 273. Page, I. H., *Biochem. Z.*, 1930, **223**, 222.

depress the activity of the bone enzyme in rats, though not to the same extent as the administration of vitamin D.

It is known that the thyroid gland also profoundly influences calcium and phosphorus metabolism. In exophthalmic goiter the excretion of calcium and phosphorus is markedly increased and there is decalcification of bone though the level of calcium and phosphorus in the blood remains normal.[5] Kay[6] and Hunter[7] have demonstrated an increase in the phosphatase activity of the plasma in hyperthyroidism but a correlation of the increased excretion of calcium and phosphorus and the decalcification of bone with the phosphatase activity of the kidneys and bones, respectively, has not been attempted. The present study was undertaken to determine whether such a correlation exists by measuring the phosphatase content of the bones and kidneys of animals rendered thyrotoxic by injections of thyroxin.

In numerous preliminary experiments, the results coincided in general with those here reported. Two litters of pure strain, white rats were chosen, 50 and 100 days old, respectively, at the onset of the

TABLE I

No.	Sex	Wt. when killed, gm.	Wt. at start, gm.	Medication	Wt. of kidneys, gm.	Wt. of bones gm.	Kidney Phosphatase Units/gm.	Bone Phosphatase Units/gm.
Litter No. 1—50 days old.								
1	M.	78.0	74.0	0.2 mg. thyroxin daily for 10 days	.92	.71	11.7	13.1
2	M.	84.5	83.8	"	.97	.83	13.1	13.3
3	M.	79.5	80.0	"	.83	.71	13.9	16.9
4	M.	64.8	74.7	"	.74	.71	16.2	21.1
5	F.	69.4	71.3	No medication	.64	.73	17.9	15.2
6	M.	75.0	78.4	"	.72	.77	18.8	21.3
7	M.	81.0	73.8	"	.81	.76	11.8	14.2
8	F.	83.4	73.0	"	.74	.77	15.1	14.2
Litter No. 2—100 days old								
1	M.	118.8	125.0	0.4 mg. thyroxin daily for 18 days	1.30	1.09	9.2	16.8
2	F.	111.0	101.0	"	1.29	0.92	7.4	23.0
3	M.	102.0	122.0	"	1.33	0.88	4.7	23.1
4	F.	100.0	98.0	"	1.16	0.86	11.3	17.1
5	M.	106.0	111.5	"	1.19	0.88	9.7	14.9
6	M.	146.5	115.5	No medication	0.96	1.02	13.9	26.7
7	M.	174.6	124.5	"	1.18	0.88	9.7	14.9
8	F.	122.0	97.0	"	0.89	0.87	21.8	18.8
9	F.	112.0	97.0	"	0.77	0.85	35.2	27.0

[5] Aub, J. C., Bauer, W., Heath, C., and Ropes, M., *J. Clin. Invest.*, 1929, **7**, 97.

[6] Kay, H. D., *Phys. Rev.*, 1932, **12**, 384.

[7] Hunter, D., *Quart. J. Med.*, 1930, **24**, 393.

experiment. There were 17 animals in all (Table I). Approximately one-half of each litter received injections of thyroxin, while their litter mates, kept in the same environmental conditions, were used as controls. The rats in the first litter received 0.2 mg. of thyroxin intramuscularly daily for 10 days, being killed on the eleventh and twelfth days. Those in the second litter were injected with 0.4 mg. of thyroxin per day for 18 days and were killed on the nineteenth and twentieth days. The injections were all given in the thigh muscles, alternate legs being used for successive injections. The thyroxin used was the Squibb crystalline product and was freshly dissolved every few days in N/50 sodium hydroxide in concentration such that 0.1 cc. was used for each injection. The effects of the thyroxin were followed by observing the loss of weight, or failure to gain weight, of the injected animals as compared with the controls.

In the litter receiving 0.2 mg. of thyroxin for 10 days, the injected animals showed an average loss of weight of 1.4 gm. while the controls showed an average gain of 3.4 gm. In the litter which received the heavier dose of 0.4 mg. for 18 days, the treated animals lost an average of 4.9 gm., whereas the untreated controls gained an average of 23.3 gm. The animals were given the same daily rations and at the end of the experiment the treated animals had eaten all of their food while 92 gm. of food were still unconsumed in one of the cages containing controls.

The method of determining the phosphatase content of the bones and kidneys was taken from Kay[8] with certain modifications. One unit is the amount of enzyme necessary to split one milligram of inorganic phosphorus from an excess of substrate during an incubation period of 2 hours at 37°C. and a pH of 8.8. Four bones, the femora and humeri, were removed from each animal and the soft tissue down to the periosteum removed. The 2 kidneys were removed and decapsulated. The bones and kidneys were thoroughly crushed in separate flasks and weighed. Twenty parts of water were added to each flask and the flasks agitated at room temperature for 48 hours.

The extract was then centrifuged for 6 minutes at 800 revolutions per minute, the same in each case, and 0.5 cc. of the supernatant fluid was put in 5 cc. of a combined reagent and incubated for 2 hours at 37°C. Control tubes containing 0.5 cc. of the extract, some in water and others in combined reagent, were set up at the same time. The control tubes were immediately inactivated by the addition of 2 cc. of trichloracetic acid. At the end of the incubation

[8] Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 235.

period, trichloracetic acid was also added to the incubated tubes, the mixtures all filtered, and the inorganic phosphorus present determined by the method of Fiske and Subbarow.[9]

The phosphatase content of the kidneys was found to vary markedly in all the animals tested. The kidneys of the thyrotoxic animals contained from 4.7 to 16.1 units of phosphatase per gram of tissue, averaging 10.8 units. In their respective controls the phosphatase content varied from 11.8 to 35.2 units per gram of tissue with an average of 19.2 units. This suggests that thyroid reduces the phosphatase content of kidney somewhat but the significance of this is diminished by the wide variations in results. An incidental finding, although the series is small, is the absolute and relative increase in weight of the kidneys in the thyrotoxic animals.

The bones showed less variation. In the thyrotoxic animals, the phosphatase content varied from 13.1 to 23.1 units, with an average of 17.7 units; whereas their controls showed a variation from 14.2 to 27.0 units with an average of 19.6 units of phosphatase per gram of tissue. Obviously, there is no significant variation from the controls produced by the injection of large doses of thyroxin.

Summary and Conclusions. The phosphatase activity of the bones and kidneys of thyrotoxic white rats has been studied. The kidneys of the thyrotoxic animals were found to be larger than those of their controls and the phosphatase content was lower per gram of tissue on the average, but there was such a wide range of variation that the results were not conclusive in so small a series. The phosphatase content of the bones of the thyrotoxic animals was the same as that of their controls. The decalcification in thyrotoxicosis is thus probably not accompanied by a change in the phosphatase activity of the bones.

[9] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1923, **66**, 375.