

EFFECT OF ADRENALECTOMY & THYROIDECTOMY ON ALKALINE PHOSPHATASE ACTIVITY OF VARIOUS TISSUES IN RAT

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Reports regarding the effect of adrenalectomy and of cortical hormones on a number of enzymes have already appeared. Tipton (27, 28) demonstrated that the activity of cytochrome oxidase and concentrations of cytochrome C in the heart, kidney and liver were decreased in adrenalectomised rats. Sodium chloride administration could partially prevent this while adrenal cortical extracts were completely effective. Similarly Folley and Greenbaum (8, 9) observed a decrease in liver arginase activity after adrenalectomy and this was not restored. Fraenkel-Conrat *et al.*, (10), Jiminez-Diaz (12), Kutscher (18) have previously reported a marked decrease in alkaline phosphatase activity after adrenalectomy in rat, guineapig and cat. Recently Vail and Kochakian (28a) reported that the liver alkaline phosphatase is slightly decreased and kidney alkaline phosphatase some what reduced in the rat following adrenalectomy. Surprisingly acid phosphatase is unaltered by these procedures.

Cohel (5) reported that thyroid hormones increase the oxidative processes in the tissues. It appears to act as a catalyst or at least as an activator of respiratory cell enzyme. This action is independent of the nervous system. Scharles (24a) observed that the thyroid hormone could alter the hydrolytic enzymes in the cell. Pincus (23) reported that this hormone probably serves as an intermediary between the organiser of Spenann (25) and the catalysts.

The thyroid hormone is also reported to influence the intestinal absorption and motility.

The present investigation was undertaken to study the effect of adrenalectomy and thyroidectomy on alkaline phosphatase activity in the intestines and other tissues with a view to find out the part played by these endocrine glands in influencing alkaline phosphatase activity in various tissues.

EXPERIMENTAL

Twenty albino rats weighing approximately 100 grams belonging to either sex were used for this investigation. They were divided into different groups as under :—

- I. Normal rats on a synthetic diet,
- II. Thyroidectomised rats on synthetic diet,
- III. Adrenalectomised rats on synthetic diet,
- IV. Adrenalectomised rats on synthetic diet receiving per corten (desoxy-corticosterone acetate).

Intestines (Jejunum close to duodenum), liver and kidneys were removed from the animals in groups III and IV after a period of twenty four hours after adrenalectomy. Tissues including adrenals from the animals in group II were taken 22-30 days after thyroidectomy. The animals in all cases were killed by stunning.

All the tissues were fixed in three changes of cold acetone for 24 hours, cleared in xylol and impregnated with paraffin at 56°C. Sections were cut at a thickness of about 5 microns.

The histological technique employed for demonstrating the alkaline phosphatase was a combination of Gomori (11) and Kabat and Furth (16) as described by Lillie (20). Sodium beta glycerophosphate was used as a substrate. No counter stain was used. Slides from each group were incubated for a period of $\frac{1}{2}$, 2 and 16 hours.

RESULTS

Group I. Alkaline phosphatase reaction in the intestine, liver, suprarenal and kidney in the normal animals was similar to that reported by previous workers.

Group II. Compared to the normals where alkaline phosphatase reaction in some cases was so intense that the structural details could not be made out after half hour incubation (fig. 1); the animals in this group gave only a moderate reaction mainly confined at the villi and only slight reaction at the crypts (fig. 4). With further incubation there was more marked reaction at the villi and crypts and also positive reaction was seen elsewhere.

LIVER—No reaction was observed in half hour sections. After two hours of incubation slight positive reaction spread all over the section, similar to that observed after half hour of incubation in normal was seen. Results after sixteen hours of incubation were also similar to normal.

KIDNEY—Reaction was similar to normal (fig. 3) but slightly less marked than that observed in the normals on half hours incubation (fig. 6). Progressively marked reaction after 2 and 16 hours was observed. No reaction in medulla was seen after 16 hours.

SUPRARENAL—Alkaline phosphatase reaction was negative after half hour of incubation (fig. 5). While in the normal alkaline phosphatase reaction was observed at the capsule, zona glomerulosa and also in the middle of zona fasciculata (Fig. 2).



Fig. 1. Alkaline phosphatase reaction in jejunum of normal rat. ($\frac{1}{2}$ hour incubation) $\times 300$ (approx).



Fig. 2. Alkaline phosphatase reaction in Suprarenal of normal rat. ($\frac{1}{2}$ hour incubation) $\times 300$ (approx).



Fig. 3. Alkaline phosphatase reaction in kidney of normal rat. ($\frac{1}{2}$ hour incubation) $\times 300$ (approx).



Fig. 4. Alkaline phosphatase reaction in jejunum of thyroidectomized rat. ($\frac{1}{2}$ hour incubation) $\times 300$ (approx).



Fig. 5. Alkaline phosphatase reaction in Suprarenal of thyroidectomised rat. ($\frac{1}{2}$ hour incubation) \times 300 (appro).

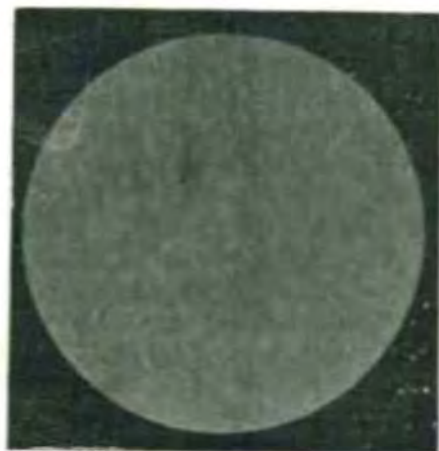


Fig. 6. Alkaline phosphatase reaction in kidney of thyroidectomised rat. ($\frac{1}{2}$ hour incubation) \times 300 (appro)



Fig. 7. Alkaline phosphatase reaction in jejunum of adrenalectomised rat. ($\frac{1}{2}$ hour incubation) \times 300 (appro).



Fig. 8. Alkaline phosphatase reaction in jejunum of adrenalectomised rat receiving Desoxycorticosterone acetate. ($\frac{1}{2}$ hour incubation) \times 300 (appro).

Analysis of the results shows that alkaline phosphatase activity in the intestine, liver, kidney and suprarenal is decreased after thyroidectomy but it is not abolished. This can be made out when half hour sections are compared with those of the normal animals. 2 and 16 hours sections in all cases did not show any marked difference in the reaction. Decreased reaction after half hours incubation was uniformly observed in all the animals of this group. One animal was sacrificed 8 days after thyroidectomy and in this slightly more reaction was observed after the same period of incubation.

Group III. After Adrenalectomy—(24 hours after).

INTESTINES—In the intestine unlike that in the normal only a moderate reaction was located at the villi after half hour incubation (fig. 7). After 2 hours of incubation, the intestines exhibited a more marked reaction at the villi and also at the crypts. The muscular walls also gave slight positive reaction. Similar but more marked reaction was obtained after 16 hours of incubation.

LIVER—In half hour slides results similar to that after thyroidectomy were observed. Slightly positive reaction restricted to nuclei was obtained after 2 hours and similar results after 16 hours but more marked in one case.

KIDNEY—No appreciable difference as compared to normal was noticed in the enzyme activity after half hour of incubation. Intense reaction was seen in sections incubated further for 16 hours.

Group IV. Adrenalectomised animals receiving injections of 0.1 cc (0.5 mgm) of desoxycorticosterone acetate and maintained for 10-15 days.

(a) **INTESTINE**—Enzyme gave moderate reaction at the villi extending to some portion on the crypts after half hour incubation (fig. 8). After two hours moderate reaction at the villi and at the crypts was noticed. Further incubation gave marked reaction spread almost all over.

(b) **LIVER**—Alkaline phosphatase reaction similar to that in normals was noticed.

(c) **KIDNEY**—No appreciable difference in the results in this group and that in normals and in Group III was observed.

On analysis it would be observed that in adrenalectomised animals receiving saline and Percorten injection of 0.5 mgm., reaction somewhat similar to that in the normal animals was maintained.

DISCUSSION

The influence of thyroid hormone in various physiological processes mediated by enzymes has been observed by various workers (13, 14, 15, 19 and 31). In the presence of glucose, sodium pyruvate or sodium succinate,

brain brei from thyroid treated rats has a higher oxygen uptake than brei from controls which received vitamin B₁ only (24). There is a decrease in the content of d-amino acid oxidase in the tissues of thyroidectomised rats (15). An increase in the content of alkaline phosphatase occurred in the tissues of animals maintained on adequate diet supplemented with thyroid tissue. Livers of female rats given thyroxine contained quantities of arginase comparable to those found after feeding diets high in protein or after long feeding. This did not occur in male rats (19). There is an increase in the Phosphatase content of the bone after thyroxine administration (31).

In the present investigation it has been observed that thyroid influences alkaline phosphatase activity of liver, kidney and suprarenal. The results of this investigation are similar to those of Williams in that there occurs a decrease in alkaline phosphatase after thyroidectomy. It was further observed that time interval after thyroidectomy is an important factor in influencing these reactions. This is probably due to prolonged effects of existing thyroxine in the animal.

There is a marked decrease in alkaline phosphatase activity in rat after adrenalectomy (10, 12, 17, 18 and 28a). Others have shown that adrenal extracts and corticosterone cause a decrease in phosphatase content of rats femur although Desoxy-corticosterone acetate produced an increase (31). In the present studies there was a slight decrease only in the alkaline phosphatase activity in the intestine where the phosphatase reaction was confined mainly at the surface of the villi. In the liver and the kidney tissues no appreciable difference in alkaline phosphatase reaction was obtained as compared to those of normals. These results are not in agreement with those of previous workers with regard to alkaline phosphatase in liver and kidneys. It is difficult to explain this discrepancy but possibly it may be due to the studies having been made at different time intervals after adrenalectomy. The present studies were probably made at an earlier stage in which case it could be concluded that alkaline phosphatase activity of the intestine is the first to be affected. Administration of percorten parentally increased the alkaline phosphatase reaction in the intestines to normal level. Adrenal cortex may be influencing the alkaline phosphatase activity of the intestine through its influence on water and electrolyte metabolism.

Adrenal cortex controls the phosphorylation which is involved in the utilization of glucose, fats, vitamin B₁ and B₂. Adrenal cortex which has been observed to influence the alkaline phosphatase activity in the intestine may therefore also be controlling the absorption of the constituents pointed out by Verzar. Claims of phosphorylation have however not been supported by subsequent workers (2,3,7,21,22), the decreased rate of Glucose absorption being dependent upon the salt balance in the animals (1,4). It has been

proved by using radioactive phosphorus that the incorporation of radioactive phosphorus into phospholipid molecule is not affected in adrenalectomized rats (26).

Adrenal cortex may be influencing the alkaline phosphatase activity in intestine but the part played by it in phosphorylation does not appear to be of much significance.

SUMMARY.

Studies of the effects of thyroidectomy and adrenalectomy on alkaline phosphatase activity of intestine, liver, suprarenal and kidney were undertaken in rats. It was observed that alkaline phosphatase activity is decreased in all these organs when examined histologically 22 to 30 days after thyroidectomy.

After adrenalectomy the alkaline phosphatase reaction in the intestine (jejunum) was moderate and restricted at the surface of villi. In liver and kidney, on the other hand, no appreciable difference as compared to normal was noticed. Percorten injection restored the reaction in the intestine to that in the normal animals.

The significance of these reactions has been discussed.

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