Serum enzyme disturbances in thyrotoxicosis and myxoedema[1]

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The serum activities of at least eight different enzymes have been shown to be influenced by thyroid functional status in humans, consistent variations having been reported in cases of cretinism, juvenile hypothyroidism, thyrotoxicosis and myxoedema. While some of the reported changes often have proved to be quite spectacular, serum enzyme studies in general have not found any great application in the diagnosis or monitoring of thyroid dysfunction, there being a range of other much more specific and sensitive tests available. Nevertheless, these phenomena have attracted considerable attention over the last twenty years, since it seemed likely that they reflect upon the metabolic disturbances of thyroid disease and thereby possibly upon the mode of action of thyroid hormones. In retrospect, however, it has proved very difficult to account for these serum enzyme activity changes in terms of detailed adjustments to intracellular metabolic pathways, not least perhaps because too many enzymes of totally different biochemical roles have been shown to be disturbed in too similar a fashion.

Common abnormalities

Table 1 lists important serum enzyme activity changes that have been noted (and by whom) in cases of hypothyroidism, and Table 2 similarly lists those reported in association with thyrotoxicosis. It is easy to see from these tables why hypothyroidism has attracted the greater attention, being associated with numerous serum enzyme elevations including aldolase, creatine kinase, the transaminases and lactate dehydrogenase. The disturbances reported in thyrotoxicosis generally have been fewer, less spectacular and more varied insofar as significant lowering of some enzymes has proved as common as elevation of others. Undoubtedly creatine kinase (CK), with its elevation in myxoedema and significant depletion in thyrotoxicosis, has been the enzyme which has attracted the greatest attention of all. This arises partly from its muscular origin – and muscular function has long been a matter of interest in thyroid disease – but mainly because it has been shown to be the enzyme whose serum activity is most markedly and consistently elevated in hypothyroidism.

Creatine kinase and adenylate kinase

It is the behaviour of creatine kinase compared with that of adenylate kinase in thyrotoxicosis and myxoedema which first stimulated my interest in this field. Adenylate kinase (AK), formerly known as myokinase, is a widely distributed enzyme, but like creatine kinase its richest source is skeletal muscle. Also like CK serum, AK has been shown to rise in association with myopathies (Kleine & Chlond 1966) and after myocardial infarction (Schreiber 1964, Lehmann *et al.* 1966), although the changes are less dramatic.

In 1971 we estimated the serum activities of the two enzymes in normal individuals after exercise, in patients who had suffered myocardial infarctions, in cases of myositis and in groups

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Table 1. Serum enzyme disturbances in myxoedema. The activities of these enzymes have been shown to rise in hypothyroidism

Enzymes affected	References
Aldolase	Sibley & Fleisher (1954)
Aspartate transaminase	Della Santa (1962)
Alanine transaminase	Della Santa (1962)
Malate dehydrogenase	Lieberthal *et al.* (1963)
Creatine kinase	Graig & Ross (1963)
Lactate dehydrogenase	Fleisher *et al.* (1965)
Hydroxybutyrate dehydrogenase	Maclean *et al.* (1968)

Table 2. Serum enzyme disturbances in thyrotoxicosis

Enzymes showing raised activity	References	Enzymes showing reduced activity	References
Malate dehydrogenase	Lieberthal *et al.* (1963)	Creatine kinase	Fleisher *et al.* (1965)
Adenylate kinase	Doran & Wilkinson (1971)	Lactate dehydrogenase	Fleisher *et al.* (1965)

of thyrotoxic and myxoedematous individuals (Doran & Wilkinson 1971). Figure 1 summarizes the results of these determinations which confirmed previous reports regarding myopathy and acute damage to muscle and myocardium, but showed that AK behaved in an almost exactly opposite manner to CK in thyroid disease, being significantly elevated in thyrotoxicosis yet unaltered in myxoedema. We have been at a loss to explain this divergence, particularly in thyrotoxicosis, although the answer might lie in the known effects of hyperthyroidism on skeletal muscle and in the subcellular location of the two enzymes. Marked loss of muscle bulk is a well known feature of thyrotoxicosis and this could well account for the lowering of serum CK activity. It may be argued from this that AK activity could be expected to fall as well, but it is important to note that the subcellular distributions of the enzymes are not the same, CK being mostly cytosolic while AK is predominantly mitochondrial. There is evidence that thyroid hormones promote pronounced mitochondrial proliferation in muscle as well as induction of higher levels of enzyme per unit of mitochondrion (Gustafson *et al.* 1965); the elevation of serum AK might simply be a reflection of this phenomenon.

Myxoedema: typical patterns of abnormality

Figure 2 summarizes the distribution of the activities of seven different enzymes measured in a group of 26 myxoedematous patients (Doran & Wilkinson 1975). The changes confirm the reports already referred to in Table 1, particularly regarding CK, and also demonstrate something that had not been described previously: that serum amylase activities tend to rise as well. A general question that arises concerns the rapidity with which these enzyme elevations develop in myxoedema. The evidence is in favour of a fairly slow process, the patient needing to be in a hypothyroid state for some while before the spectrum demonstrated in Figure 2 can be expected. In order to confirm this, I recently studied the serum CK levels in a group of 20 individuals who were considered to be in the early stages of hypothyroidism. This classification was made on both clinical grounds and on the basis of the results of their thyroid function tests. All showed some elevation of serum thyroid-stimulating hormone, although in two-thirds of the cases this was only minimal (5–10 mu/l). In addition, one case in three showed either a reduced thyroxine (T_4) or triiodothyronine (T_3) level as well. However, only one of these 20 patients had a raised serum CK; no other enzymes were assayed.

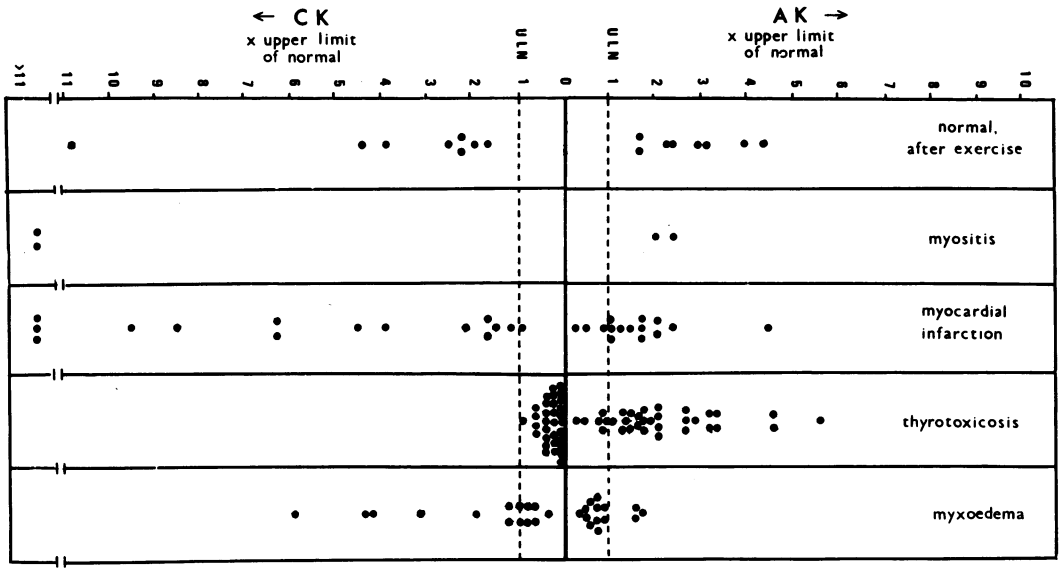
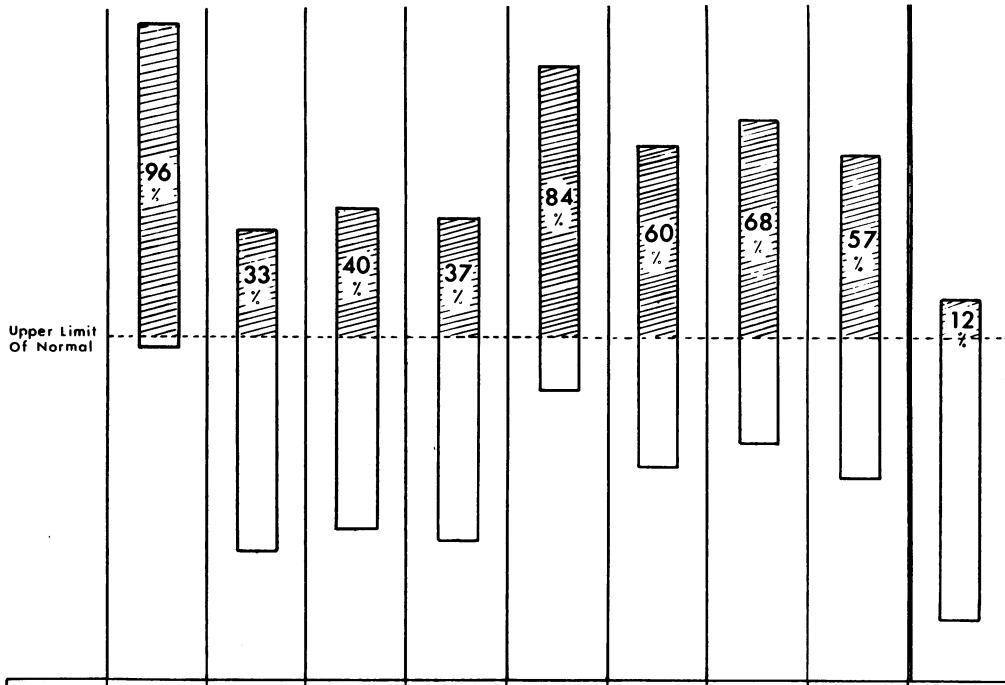


Figure 1. Serum creatine kinase (CK) and adenylate kinase (AK) activities in normal subjects after exercise and in patients with myocardial infarction, myositis, thyrotoxicosis and myxoedema. The broken lines represent the upper limits of the normal ranges (ULN)



ENZYME	CK	AK	HBD	LD	AST	ALT	ALD	AMYL	AMYL EUTHYROID
MEAN ACTIVITY	410 I.U./L	18 I.U./L	159 I.U./L	244. I.U./L	69 I.U./L	46 I.U./L	3.8 I.U./L	325 I.U./L	223 I.U./L
RANGE	96 - 1769	0 - 54	30 - 404	76 - 556	11 - 172	13 - 129	1.4 - 9.0	72 - 554	99 - 337
NUMBER	26	18	16	16	25	25	19	25	26

Figure 2. A summary of the distributions of activities of serum creatine kinase (CK), adenylate kinase (AK), hydroxybutyrate dehydrogenase (HBD), aspartate transaminase (AST), alanine transaminase (ALT), aldolase (ALD) and amylase (AMYL) in a group of myxoedematous patients. Shaded areas represent the proportion of elevated values in each case. A control group of amylases in euthyroid individuals is included for comparison

Tissue origins

The tissue origins of some of the excess enzymes circulating in myxoedema is also a subject which has received considerable attention. My investigations into the isoenzyme composition of the circulating CK using starch-gel electrophoresis have shown, as have others, that the enzyme derives almost entirely from skeletal muscle; the MM band only being demonstrable in virtually all cases, with no evidence of significant quantities of the MB or BB fractions (Doran & Wilkinson 1975).

Although less specific information is available, increased leakage from muscle would seem to be a likely explanation for the elevations of aldolase, the transaminases, and lactate and malate dehydrogenase as well. Furthermore, my studies of the behaviour of some enzymes (apart from amylase) whose serum activities are not considered to reflect upon muscle disease, including isocitrate dehydrogenase, 5'nucleotidase and gamma-glutamyl transferase, have shown that they do not rise significantly in myxoedema. However, this evidence of a largely skeletal muscle origin for many of the enzymes involved may not be considered very surprising since muscle constitutes by far the largest proportion of all the body's soft tissues.

Possible explanations

The question of how these serum enzyme elevations come about remains largely unanswered. The simplest and most attractive theory is that hypothyroidism causes increased rates of leakage of enzymes from cells, probably involving other tissues as well as muscle, and that this may well be coupled with a decreased rate of enzyme clearance from the circulation. The implication is that the phenomenon is nonspecific in that it increases the permeability of the plasma membranes of many types of body cells, permitting faster leakage of a variety of enzymes – the cytosolic being most readily affected.

The *in vitro* experiments of Robinson & Wilkinson (1974, 1975) demonstrated that adenosine triphosphate (ATP) levels significantly influence the rate of leakage of enzymes from cells, and a general hypothesis of depletion of cellular high energy phosphates in the hypometabolic state of myxoedema would seem a simple explanation for the apparent increased cell membrane permeability. In looking for some specific means whereby cell membrane ATP levels could be reduced in myxoedema, I have recently examined the direct *in vitro* effect of thyroid-stimulating hormone (TSH) upon enzyme leakage from human white blood cells. It is known that this hormone does have effects upon other tissue besides thyroid cells (e.g. adipose tissue: Engel *et al.* 1957) and acts by the stimulation of adenyl cyclase, a process which could conceivably bring about depletion of cell membrane ATP.

Saline-washed fresh human white blood cells (mixed populations, average count $30 \times 10^6/1$) were suspended in a physiological medium (improved Krebs-Ringer II) and incubated at 37°C. After one hour, bovine TSH was added to the test suspensions to produce a concentration of 100 mu/l. The rate of leakage of lactate dehydrogenase from the cells was monitored both prior to the hormone addition and for up to six hours afterwards and compared with that in control suspensions to which no hormone was added. Figure 3 shows the mean relative rates of enzyme leakage from the test cells compared with that in the controls. The experiments showed that TSH, for a short period at least, either actually enhanced the rate of leakage or reduced its decline. When the enzyme leakage rates during the first hour following TSH addition were expressed as multiples of the leakage rates during the hour preceding this addition, comparison of test ratios with their controls revealed highly significant differences ($t=3.75$, $P=<0.005$, $n=21$). Experiments are now under way to see if similar effects can be demonstrated in other tissues, particularly skeletal muscle. While great caution must be exercised in extrapolating the results of these *in vitro* experiments to the *in vivo* situation, they have suggested that TSH, circulating at high levels for long periods as commonly occurs in myxoedema, might have a widespread effect upon a variety of tissues other than the thyroid gland, bringing about increased leakage of enzymes. Even if this should be true it cannot be the only cause; if it were, the abnormalities could be expected to manifest themselves at a much earlier stage, i.e. when the TSH levels first begin to rise.

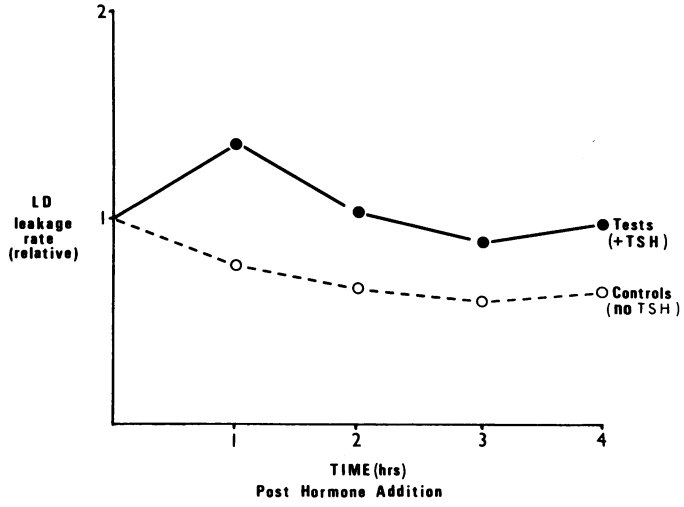


Figure 3. Mean curves showing lactate dehydrogenase (LD) leakage from human white blood cells following exposure to thyroid-stimulating hormone (TSH). The leakage rates of both tests and controls are represented as multiples of the rates recorded during the hour preceding hormone addition, these being expressed as unity

Hypothermia is another possible causative factor which has been implicated in hypothyroidism. It is well known that the reduced metabolic rates of myxoedema result in a significant fall in body temperature, and some untreated cases may decline into hypothermic coma. It has also been shown that accidental hypothermia developing in euthyroid individuals rapidly results in elevations of serum enzyme activities to give patterns indistinguishable from those seen in cases of long-standing hypothyroidism, or indeed myxoedematous hypothermic coma (Maclean *et al.* 1968, 1974). Yet hypothermia as such still explains very little in detail and I am of the opinion that hypothermia alone does not account for the apparent leakiness of cells in myxoedema.

Conclusions

It seems likely that the pattern of serum enzyme elevations seen in so many cases of myxoedema is attributable to several factors, a major one of which would appear to be some modification of the physical properties of cell membranes, an abnormality which perhaps also can be rapidly induced in the membranes of normal cells by hypothermia. This abnormality might derive from a continuous deficiency of ATP and may also involve some progressive change of chemical composition of the membranes bringing about a loss of membrane fluidity. These matters, however, must remain interesting speculations until some more positive evidence becomes available.

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Fifty years ago

R. S., MALE, aged 56. A healthy, well-nourished man, by occupation an engine driver, with a month's history of a gradually increasing tumour in the right trapezius muscle.

Past History. – The man has always been healthy. In 1919, in consequence of his daughter's illness, he had a Wassermann test, but it was negative; infection of a specific nature was, however, present on his wife's side of the family.

History of Present Illness. – About a month ago he noticed his right brace galled his shoulder and found a lump above the clavicle. This has slowly increased in size and is now rather tender to the touch.

Present Condition. – The patient, though obese, is quite healthy, except for the tumour.

In the right trapezius, and apparently adherent to the clavicle and subjacent muscles, is a hard ovoid mass about the size of a pigeon's egg. The tumour has ill-defined edges and is fixed in the muscle, though the superjacent skin is mobile and normal in appearance. There is no translucency.

X-rays show no abnormality in the bones or chest.

November 5, 1927. – Wassermann reaction negative.

The tumour appeared to be a fibro-sarcoma.

I still think it is sarcoma, though I would now wish to withdraw the prefix 'fibro-.' I do not know whether it is because of the handling it has received or due to the X-rays, but it has altered in appearance recently and become fluctuant.

Postcript. – At operation the tumour proved to be a Morant Baker's cyst, which had arisen in connexion with rheumatoid arthritis of the acromio-clavicular joint and had travelled in deep to the trapezius.

From 'Tumour of Trapezius' by P H Mitchiner MS FRCS (*Proceedings of the Royal Society of Medicine*, Clinical Section, 1927–28, **21**, 270)