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Changes in blood chemistry and liver histopathology of rabbits during experimental tetanus toxicity

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Summary

Changes in blood chemistry, especially blood glucose, were studied in rabbits developing tetanus after injection of tetanus toxin. Blood glucose levels increased significantly above control values after the animals developed tetanus. The increase in glucose level paralleled the depletion of liver glycogen, detected by means of PAS staining. The observed changes were not affected by adrenergic receptor block or catecholamine depletion by reserpine, suggesting non-mediation of the sympathetic nervous system in the response. A direct action of the toxin on the liver, initiating glycogen depletion, is being postulated.

Introduction

Patients suffering from tetanus have been shown to exhibit changes in some of the chemical constituents of their blood. The reported changes include increases in serum creatinine phosphokinase-CPK and serum transaminases-SGOT and SGPT (Bademosi, 1979; Chio-Kang & Hanna, 1980).

It has also been reported that some of these patients do develop raised blood glucose levels (Kloetzel, 1963; Bademosi, 1979) and that the glucose level also appears to correlate with the severity of the disease (Bademosi, 1979). However it has been suggested that the raised blood glucose levels observed in these patients may have been due to the use of intravenous solutions, containing glucose, during the process of treatment (Kloetzel, 1963).

In the present study, we have induced tetanus in rabbits by injecting tetanus toxin, while determining changes in the blood chemistry of these rabbits with particular emphasis on changes in blood glucose level. In addition, attempts were made to study the mechanisms involved in the blood sugar changes and this included histological examination of the liver.

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Abbreviations used: Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Periodic Acid Schiff reaction (PAS), Generalized Tetanus (GT) and Local Tetanus (LT).

Materials and methods

Experiments were carried out on thirty-two male and female rabbits weighing between 1.5 and 2.0 kg. The rabbits were inbred strains obtained from the animal house of the College of Medicine, Lagos, and were fed on normal rabbit pellets and water. Blood was obtained from the animals which were fasted for 12–14 h, by inserting a 21 G needle into the middle artery of the ear. About 6 cm^3 of blood was obtained into heparinized tubes for blood chemistry analyses. These blood samples served as the control. The rabbits were then injected, subcutaneously in the groin, with tetanus toxin (Swiss Serum and Vaccine Institute, Berne) at a dose of 2000 MLD/kg body weight. The animals developed local tetanus, characterized by rigidity of the injected limb within 18 h followed by generalized tetanus, characterized by spasms, in about 30 h. Then another 6 cm^3 of blood was taken from eight of the rabbits with generalized and six with local tetanus. All blood samples were taken in all rabbits fasted for 12–14 h. The blood analyses were carried out in the clinical chemistry laboratory of the Lagos University Teaching Hospital. The blood was analysed for

sodium (Na^+), potassium (K^+), chloride (Cl^-), bicarbonate (HCO_3^-), urea, serum creatinine, SGOT, SGPT and blood glucose. Glucose levels were determined by the glucose oxidase method.

The rabbits were killed by cervical cordotomy and portions of the liver were excised and fixed in 10% formalin for subsequent histological study. Six rabbits that were not injected with tetanus toxin also have their livers fixed for histology and these served as control for comparison with livers from the tetanus rabbits.

Histopathological examinations were carried out on two representative sections taken from constant sites in the right and left lobes of the liver in each rabbit. These were fixed in 10% formalin solution and some of our sections were fixed in cold acetic acid-formalin in 95% alcholol saturated with picric acid to assess the polarization that usually occurs after tissue fixation. The sections were then prepared for paraffin embedding. Thin (5 μ) cryostat sections were stained with haematoxylin and eosin (H&E) and periodic acid Schiff reagent without and with diastase (PAS & PASD respectively). Selected sections were also stained with Masson Trichrome and Gordon sweet reticulin reagents. The sections were examined under light microscope at both low (×10 objective) and high (×40 objective) power magnifications.

In a further study, twelve rabbits were divided into three groups of four rabbits each. The first group was given reserpine (Serpasil, CIBA) 3 mg/kg on day 1 and 1.5 mg/kg on days 2–4 (Sofola *et al.*, 1981). Tetanus toxin was then injected in these rabbits on day 3. In the second group, phenoxybenzamine (Dibenyline, Smith, Kline & French) 2 mg/kg body weight, was administered every 12 h for 2–3 days and in the third groups, propranolol (Inderal, ICI) 2 mg/kg was also given every 12 h for 2–3 days.

Tetanus was induced in all three groups of rabbits as described earlier. In these groups, control levels of blood glucose were also determined just before injecting the tetanus toxin. After the rabbits had developed generalized tetanus, samples of blood were also analysed for blood glucose concentration. In addition the livers of these rabbits were examined histologically.

Results

Blood chemistry

(a) Changes in rabbits with generalized tetanus. The experimental animals injected with tetanus toxin developed symptoms of generalized tetanus characterized by spasms. In these rabbits there were significant increases in the blood glucose level as well as level of the serum transaminases (SGPT). Blood glucose increased from average control value of 98 mg% to 175 mg% ($P < 0.001$) while SGOT increased from 38 to 68 unit/ml ($P < 0.001$) and SGPT from 45 to 62 unit/ml ($P < 0.025$). There were no significant changes ($P > 0.1$) in the other parameters measured i.e. blood urea, serum creatinine, alkaline phosphatase, sodium, potassium, chloride and bicarbonate. These results are summarized in Table 1. The control values of all the parameters are within the normal limits described for rabbits in this environment (Adewoye, 1981). The results of three rabbits whose blood haemolysed were excluded from the analysis.

(b) Effect of reserpine or sympathetic blockers on changes in blood glucose level in rabbits with generalized tetanus. Reserpinization or prior administration of either propranolol (a beta-blocker) or phenoxybenzamine (an alpha-blocker) had no significant effect on the rise in blood glucose level seen in the rabbits that developed generalized tetanus. In the reserpinized rabbits, blood glucose increased from average control value of 81 mg% to 113 mg% (60.2% increase). Similarly in the rabbits administered with propranolol, it increased from 109 mg% to 187 mg% (71.6% increase) and in those with phenoxybenzamine, it also increased from 104 to 185 mg% (77.6% increase) (Table 2). These increases were not significantly different ($P > 0.1$) from those of the rabbits with tetanus, which were not administered with any drug.

(c) Blood glucose levels in local tetanus. In four of the six rabbits studied at the stage of local tetanus, there were also increases in blood glucose level from control values. The average increase was from 99 mg% to 125 mg% (26.2% increase) which was significant ($P < 0.025$) (Table 3). However, this increase in blood glucose during local tetanus was lower than that

Table 1. Changes in blood chemistry of rabbits with generalized tetanus

Blood constituent	C	GT	n	*P*
Blood glucose (mg/100 ml)	98 ± 7	175 ± 12	7	< 0.001
SGOT (units/ml)	38 ± 4	68 ± 6	4	< 0.001
SGPT (units/ml)	45 ± 3	62 ± 3	4	< 0.25
Serum alk. phosphatase (units/ml)	6.0 ± 1.0	6.4 ± 1.2	4	N.S.
Serum creatinine (mg/100 ml)	1.05 ± 0.2	1.03 ± 0.2	5	N.S.
Blood urea (mg/100 ml)	47 ± 2	49 ± 4	6	N.S.
Serum Na^+ (mEq/l)	140 ± 1	143 ± 2	4	N.S.
Serum K^+ (mEq/l)	3.7 ± 0.2	3.9 ± 0.2	4	N.S
Serum Cl^- (mEq/l)	104 ± 1	107 ± 2	4	N.S.
Serum HCO_3^- (mEq/l)	16.8 ± 1.2	17.2 ± 1.0	4	N.S.

C = control values.
GT = values in the rabbits on developing generalized tetanus.
P = from paired *t* test.
N.S. = not significant.

Table 2. Blood glucose changes in rabbits with prior administration of reserpine, phenoxybenzamine or propranolol

Drug	Blood glucose (mg/100 ml)				
	C	GT	*n*	*P*	% change
Reserpine	113 ± 6	181 ± 9	4	< 0.001	60.2
Phenoxybenzamine	104 ± 8	185 ± 5	4	< 0.001	77.6
Propranolol	109 ± 7	187 ± 19	4	< 0.025	71.6

Legends as in Table 1.

Table 3. Changes in blood glucose levels in rabbits developing local tetanus (LT)

Rabbit No.	Blood glucose (mg/100 ml) C	T
LT_1	88	131
LT_2	109	128
LT_3	103	123
LT_4	95	118
Mean	99	125
s.e.	3.9	4.9
t-paired	5.3783	
P	< 0.025	

C = Control values.
T = Value during local tetanus.

seen in the rabbits developing generalized tetanus ($P < 0.05$). Two of the rabbits were allowed to progress to the stage of generalized tetanus and blood glucose levels were determined. In one of these rabbits, the control glucose level was 109 mg%, rose to 128 mg% at the stage of local tetanus and to 191 mg% at generalized tetanus. The corresponding values in the second rabbit were 95 mg% (control), 118 mg% (local tetanus) and 175 mg% (generalized tetanus) respectively.

Histopathological changes in the liver

(a) Rabbits with generalized tetanus. The main findings on light microscopy with H&E stain were acute congestion of the sinusoids and ballooning degeneration of the hepatocytes in random fashion (zone I–III focally (Fig. 1)). PAS stain shows scanty granules mainly in hepatocytes in zone III and focally in those in zone II (Fig. 2a), when compared with normal control rabbits (Fig. 2b). The control rabbits which were not administered with the toxin showed positive glycogen granules in all hepatocytes. The granules were removed by diastase confirming them to be glycogen. The PAS negative cells were mainly in zones II and I (midzonal and periportal) while those around the central vein were least affected.

(b) Rabbits with local tetanus. H&E stain did not show any abnormality. However, special stain for glycogen (PAS ± D) revealed patchy loss of granules in the hepatocytes. The percentage of hepatocytes staining negatively in the rabbits with local tetanus was fewer than in those with generalized tetanus (Fig. 2c). The two other rabbits that were left to progress to generalized tetanus showed the same degree of glycogen depletion as in the earlier series of rabbits with generalized tetanus.

Effects on muscle glycogen

Samples of skeletal muscle from the uninjected thigh, of four rabbits that developed generalized tetanus, were also stained with PAS. Histological examination did not reveal any differences in muscle glycogen content between the tetanus and the control rabbits.

Discussion

The results of the present experiments have established that in rabbits developing tetanus after administration of the toxin, the fasting blood glucose level is elevated. Similarly, there are also increases in the serum transaminases. The increase in SGOT and SGPT have been reported earlier and there are suggestions that these may be due to damage to the liver or skeletal muscle (Ghanem *et al.*, 1973; Chio-Kang & Hanna, 1981).

Although blood glucose has been shown to be increased in human patients with tetanus (Kloetzel, 1963; Bademosi, 1979), there has been no report to our knowledge, of any study of the probable mechanisms involved. In fact in one study the use of intravenous glucose during therapy in those patients has been implicated as the cause of the elevated blood level (Kloetzel, 1963). However since we have not given added glucose to these rabbits, this source cannot be an explanation. A mechanism which was envisaged was that of increased sympathetic activity. Increased activity of the sympathetic nervous system in tetanus patients has been well described (Kerr *et al.*, 1968; Corbett *et al.*, 1969). This has also been demonstrated in experimental animals injected with tetanus toxin and is characterized by an increase in blood pressure, heart rate and cardiac contractility (Odusote & Sofola, 1976; Sofola & Odusote,

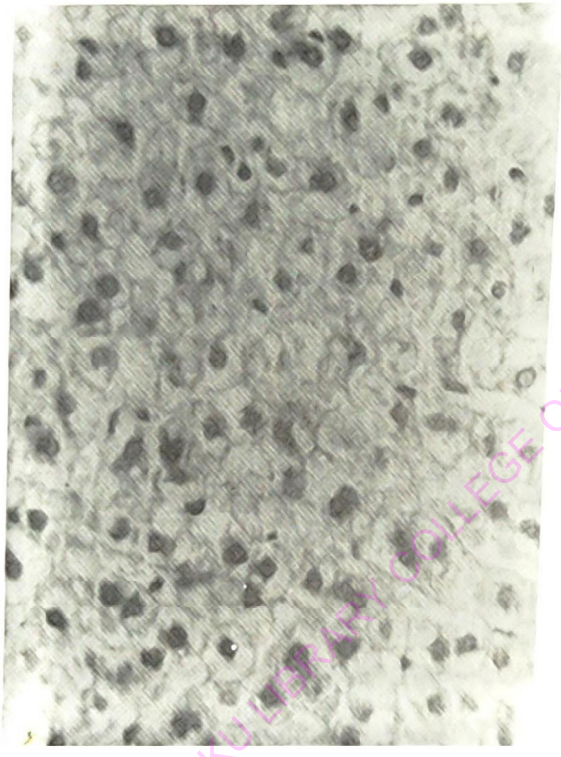

Fig. 1. Section of liver showing ballooned hepatocytes with rarefaction of the cytoplasm (H&E stain ×100).

1982). Increased sympathetic activity liberates catecholamines which are glycogenolytic (Burn, 1975). However, prior depletion of body catecholamines with reserpine or adrenergic block with propranolol or phenoxybenzamine did not appreciably alter the hyperglycaemic effect of tetanus toxicity in these rabbits. Although glycogenolysis is a beta-adrenergic effect in most species, in the rabbit it is an alpha-adrenergic effect (Burn, 1975), hence the use of phenoxybenzamine. The lack of effect of all these agents practically eliminates the mediation of the increase in blood glucose by increased sympathetic activity.

The present study has shown that during tetanus toxicity, the increase in blood glucose is associated with glycogen depletion in the liver. This is further shown by the present observation that in local tetanus, the increase in blood glucose level and hepatic glycogen depletion is not as marked as those seen in rabbits with generalized tetanus.

This probably represents a progression of the effects of tetanus toxicity with severity of the disease. It is therefore reasonable to assume that the hyperglycaemia seen in tetanus toxicity is due largely to hepatic glycogenolysis. The mechanism for this is not very clear. What we have observed are that hepatocytes around zones II and I i.e. mid-zonal and periportal are largely affected while those in zone III (around the central vein) are least affected. This may suggest a humoral factor which is diffusing from the arterial blood to affect the surrounding cells i.e. periportal cells. What this factor is, we are not able to ascertain as yet. In order to observe if the so-called 'streaming' effect (polarization) of glycogen through the cell with the fixative was significant in our experiments, we tried the method of Lison and Vokaer (1949) with

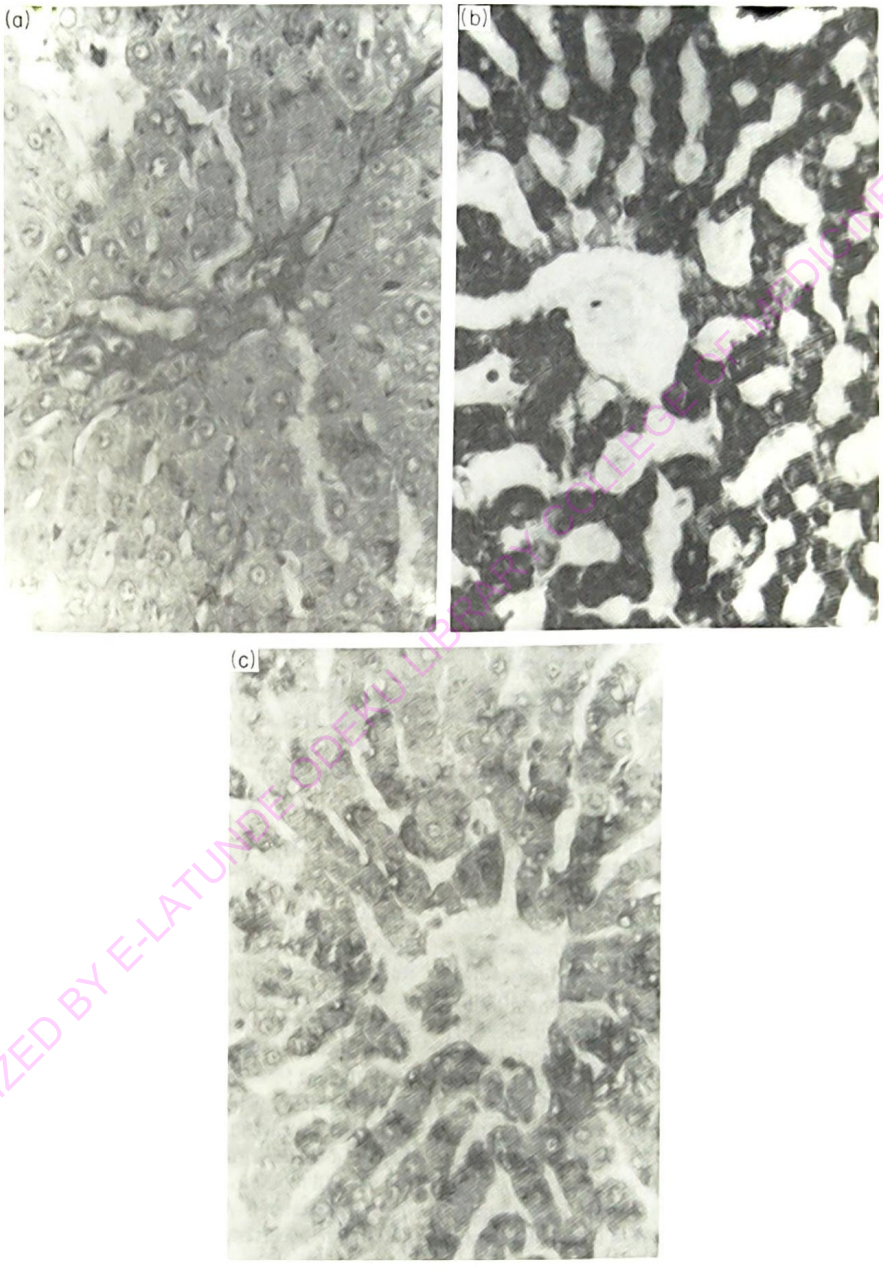

Fig. 2. (a) Section of liver from a rabbit with generalized tetanus. Positive glycogen granules are seen in only a few hepatocytes (PAS ×400); (b) Section of liver from control rabbit showing abundant glycogen granules in all the hepatocytes. These granules were removed by diastase digestion. (c) Section of liver from rabbit with local tetanus showing moderate depletion of glycogen granules (PAS ×400).

fixation in cold acetic acid-formalin in 95% alcohol saturated with picric acid with some of our sections. We did not observe any appreciable difference in 'streaming' when compared with our thin cryostat sections fixed in formalin. We believe that our method, with the precautions mentioned above, gives the glycogen distribution in the rabbit liver as accurately as possible. As has been emphasized by Ockerman (1967), tissue staining of glycogen at best is a semi-quantitative method.

There are other possibilities apart from hepatic glycogenolysis that may be contributory to the hyperglycaemia. One of them is the fact that spasms may lead to increased glucose release by the muscles. However, this is not likely to be the cause since mild to moderate levels of muscle contractions invariably are associated more with glucose uptake by the muscles and in severe cases may lead to an actual fall in blood glucose levels (Wahren, 1977). In addition, the observation that the hyperglycaemia and hepatic glycogen depletion occurs in local tetanus, where spasms have not set in, will not support the theory of increased muscular activity. Lastly, our histological findings did not indicate muscle glycogen breakdown. Another possibility is that tetanus, being a stressful condition may lead to a rise in serum cortisol levels which can increase blood glucose by gluconeogenesis. However, this is not likely to be associated with glycogen depletion in the liver, although it may remain a contributory factor to the observed hyperglycaemia, but this has not been proved in the present experiments.

In conclusion we have shown that in rabbits developing tetanus after administration of tetanus toxin, blood glucose level is elevated and this is associated with hepatic glycogen depletion suggesting this to be the main source of the extra glucose in the blood. The mechanism by which hepatic glycogen is depleted remains to be elucidated.

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