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A STUDY ON THE FUNCTION OF SOME SUBCELLULAR SYSTEMS OF THE SHEEP MYOCARDIUM DURING GOUSIEKTE. II. THE CONTRACTILE PROTEIN SYSTEM*

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ABSTRACT

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Two groups of Merino sheep were intoxicated separately and at different times with "gousiektebossie" *(Pachystigma pygmaeum)* until definite symptoms of heart failure were auscultated. Cardiectomy was carried out and some ventricular muscle from 1 group was stored in 50% glycerol at –20 °C for about 4 months. Natural actomyosin (n-actomyosin) was subsequently extracted and tested for magnesium, calcium and adenosine triphosphate (ATP)-dependent adenosine triphosphatase (ATP-ase) activity as well as for superprecipitation characteristics. Muscle strips were taken from the other group and stored for 2 weeks in 50% glycerol at –20 °C, whereafter it was analysed for an isometric tension-calcium response.

The data showed no difference between gousiekte and control sheep in the sensitivity of the contractile system to the activating effect of calcium ions with respect to isometric tension development. A significant reduction of the magnesium dependent ATP-ase was found for gousiekte n-actomyosin in either the presence or absence of calcium ions. A depressed sensitivity for this enzyme to increasing concentrations of ATP in comparison to controls was also found ([ATP] < 1 mM, [$MgCl_2$] = 1 mM). No significant difference could be detected in the sensitivity of the n-actomyosin:ATP-ase system to magnesium. n-Actomyosin:ATP-ase of gousiekte hearts revealed a depressed sensitivity to calcium ions. Gousiekte n-actomyosin also showed a significant depression in the rate of superprecipitation with a concomitant increase in the duration of the clearing phase.

We conclude from these observations that a definite biochemical lesion is induced in the contractile proteins of heart muscle obtained from sheep intoxicated with "gousiektebossie" at the stage of cardiac failure. This condition is characterized by abnormal superprecipitation characteristics and a depressed n-actomyosin:ATP-ase activity, showing a reduced sensitivity to the activating effect of calcium ions.

INTRODUCTION

Gousiekte, described as a toxic primary congestive cardiomyopathy (McKinney, 1974; Pretorius, Terblanché, Van der Walt & Van Ryssen, 1973), is caused by the ingestion of 5 different members of the plant family, Rubiaceae (Hurter, Naudé, Adelaar, Smit & Codd, 1972). It appears only among ruminants and is characterized by sudden death after a latent period of about 2–6 weeks (Pretorius *et al*, 1973). Definite symptoms of heart failure concomitant with dilatation of the ventricles were observed (Theiler, Du Toit & Mitchell, 1923; Pretorius & Terblanché, 1967). The slight cardiac hypertrophy noticed by Pretorius *et al.* (1973) was corroborated in a later report by Snyman, Van der Walt & Pretorius (1982), who, however, found no hypertrophy after allowance was made for the oedema found in the heart muscle of gousiekte sheep.

With respect to the intracellular metabolism of the heart muscle from gousiekte sheep, a depressed uptake of calcium ions by isolated fragments of sarcoplasmic reticulum was reported (Pretorius *et al.*, 1973). In another paper (Snyman *et al.*, 1982), some aspects on the energy metabolism studied showed a decreased content of high energy phosphate, an increase in the anaerobic energy state noticeable from the increase in lactate and adenosine triphosphate:creatine phosphate (ATP:CrP), and a reduced uptake of oxygen by isolated mitochondria. These deviations were interpreted as being indicative of a decreased aerobic energy production and were reported to be essentially in agreement with observations made on some other types of heart failure.

It has been postulated that, in molecular terms, at least 2 classes of heart failure can be distinguished, namely, that in which the defect lay in the generation of ATP and that in which the defect lay in the utilization of ATP (Olson & Schwartz, 1951). The high-energy phosphate stores in the heart thus reflect a balance between energy production and energy utilization. Seeing that an imbalance has been shown for gousiekte hearts, which could a least partially be attributed to a defective aerobic energy production (Snyman *et al.*, 1982), it is also of importance to evaluate the contribution of the energy utilization system to the lowered concentration of high energy phosphate stores.

The function of the contractile proteins was studied, as it plays, quantitatively seen, an important role in the utilization of energy. It also plays a key role in the process of muscle contraction and any defect, irrespective of how the energy utilization property is influenced, would seriously influence the contractile process.

In this study the superprecipitation phenomenon, which is regarded as a test tube model of muscle contraction and ATP-ase activity, which is referred to as a biochemical manifestation of muscle contraction (Hozumi & Hotta, 1977), were studied with n-actomyosin* isolated from the hearts of control and gousiekte sheep. Determinations of the sensitivity of n-actomyosin:ATP-ase with respect to calcium, magnesium and ATP were also made. The physical sensitivity of the contractile system to calcium ions was investigated by means of the isometric tension development of glycerol-extracted muscle fibres. A depressed function of the contractile proteins was indicated for the heart muscle of gousiekte sheep.

MATERIALS AND METHODS

Induction of experimental gousiekte

The hearts of the animals used for investigation of the energy production system during gousiekte (Snyman *et al.*, 1982) were also used to study the contractile protein system during gousiekte as reported in this paper.

As described in the former paper (Snyman *et al.*, 1982), the animals (Merino wethers) were intoxicated with *Pachystigma pygmaeum* at a rate of 25 g of wet material/kg body mass/day, until gousiekte occurred. Gousiekte was diagnosed when one or more of the following symptoms of heart failure occurred: tachycardia (>100), a gallop rhythm and/or a systolic murmur. After gousiekte was diagnosed, the sheep were killed while anaesthetized by rapid cardiectomy. The detail of the induction of experimental gousiekte is described in the former paper (Snyman *et al.*, 1982).

* The research was done while the senior author was in the service of the Potchefstroom University for Christian Higher Education

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* The contractile protein complex, consisting of actin, myosin, tropomyosin and troponin

For the purpose of this paper, the treatments described in the former paper (Snyman *et al.*, 1982) were renumbered. Groups II and III, the sheep of which were intoxicated with wet material, were renumbered as Groups I and II for the purpose of this paper. Group IV, the sheep of which were dosed with 200–300 g of dried material for 11–14 days and killed without any prominent signs of gousiekte, was renumbered as Group III in this paper.

Sampling

For sampling, the sheep were anaesthetized with sodium pentobarbital while being artificially respirated with oxygen, and killed by rapid cardiectomy as described in a preceding paper (Snyman *et al.*, 1982). Strips of papillary muscle from Groups I and III were glycerinated, as described by Henry, Ahumada, Friedman & Sobel (1972). In the present experiment, however, the strips, before being used for calcium-isometric tension response studies were stored in the 50% glycerol medium at –20 °C for 2 weeks. A portion of the ventricular muscle, obtained from the hearts of Group II, was cut into small pieces and glycerinated in the same way, except that it was stored in the 50% glycerol medium at –20 °C for about 4 months before being used for ATP-ase and superprecipitation studies of n-actomyosin.

Calcium-isometric tension response

A muscle strip of about 0,4 mm or less in diameter and about 4–6 mm in length was cut from the glycerinated strip and mounted vertically in a reaction bath. The lower end was connected to a stainless steel hook at the bottom of the reaction bath and the upper end to the arm of a Stratham force transducer, mounted on a micromanipulator. The connections were made by using silk pulled several times through a piece of beeswax to give it rigidity. The mounted muscle strip was covered with 3 mℓ of reaction medium kept at 25 °C by water circulating through a surrounding jacket from a thermostated waterbath. The reaction medium could be removed by means of an outlet at the bottom of the reaction bath. New medium had to be pipetted in from the top opening of the bath. The contraction and relaxation media used were described by Henry *et al.* (1972). To obtain the desired concentrations of EGTA-buffered calcium in the contraction media, calcium and non-calcium containing contraction media were mixed as described by Julian (1971).

The muscle strip was mounted and kept in relaxation medium for about 1 h. The medium was then replaced with a fresh supply, and the muscle strip stretched by adjusting the micromanipulator until the generation of some passive tension was indicated by the recorder pen. After the tension, which normally took about half an hour, had stabilized, the relaxation medium was replaced by contraction medium containing a pCa value of 5,20. A maximum isometric contraction was thus effected. This contraction was maintained for about half an hour, after which the glycerinated muscle strip was again relaxed for about an hour to ensure complete relaxation. The Ca-isometric tension response was then studied by tension development at the following successive calcium concentrations: pCa=6,95; 6,72; 6,51; 6,09; 5,90; 5,49 and 5,20. This was carried out without the intermittent relaxation steps. The isometric tension height, which was recorded at each calcium concentration, was expressed as a percentage of the maximum isometric tension height obtained, usually at pCa=5,49 or 5,20.

Extraction of n-actomyosin

The glycerinated muscle was washed 4 times with 10 mM imidazole solution (6 °C), with 10 minutes standing periods in between. n-Actomyosin was then extracted according to the method of Merin, Kumazawa & Honig (1974). This method was chosen to limit any possible alterations in activity to a minimum (Merin *et al.*, 1974) and to obtain a preparation which represented the *in vivo* situation more closely. Honig (1968) found that the rapid extraction technique, which essentially corresponds to the one used in this study, regularly yields myosin B responsive to catecholamine analogs, while drugs modified the behaviour of only half the preparations obtained by prolonged extraction. By using preliminary glycerol extraction and a brief extraction of n-actomyosin together with the addition of sodium azide, the contribution of ATP-ases from membrane systems could be regarded as being eliminated.

The isolated n-actomyosin was brought into suspension in the appropriate medium by using a Potter-Elv. homogenizer with a loose-fitting pestle.

Superprecipitation

Superprecipitation studies with n-actomyosin were carried out according to the optical density method introduced by Ebashi (1961). Stirring was applied and the OD change was followed at constant temperature (23 °C) (Yasui & Watanabe, 1965) in a Beckman model DU spectrophotometer at 660 nm.

The experiments were performed in a reaction mixture composed of 100 mM KCl, 1 mM $MgCl_2$, 1 mM ATP, 4 mM EGTA-(Ca) (pCa=5,2), 5 mM sodium azide and 20 mM imidazole, pH 7,0. A nearly constant ratio of actomyosin to incubation medium, giving a protein concentration of about 1,0 mg/mℓ, was used for all experiments.

n-Actomyosin:ATP-ase

The ATP-ase experiments were conducted in a reaction medium having the same composition as was used for superprecipitation studies, except that a $CaCl_2$-EGTA containing medium was used only for the Ca-dependent studies (pCa=6,5 and 5,2). Different concentrations of ATP (0,1 mM; 0,2 mM; 0,4 mM; 0,6 mM; 0,8 mM and 1,0 mM) and $MgCl_2$ (0,2 mM; 0,4 mM; 0,6 mM; 0,8 mM; and 1,0 mM) were used in studying the ATP and magnesium dependency of n-actomyosin: ATP-ase. The n-actomyosin concentration in all cases was about 0,25 mg/mℓ.

The incubations were done in stoppered test-tubes fixed in a shaking water-bath at 37 °C. The reaction was initiated by mixing 5 mℓ of the enzyme suspension together with the substrate. The reaction was stopped by adding 1 mℓ of 40% TCA at 0°C after exactly 15 minutes of incubation. The test-tubes were immediately immersed in ice for about 15 minutes. Then the protein precipitate was separated by centrifugation at 0 °C and the inorganic phosphate in the supernatant determined by the method of Fiske & Subbarow (1925) as applied by Furchgott & De Gubareff (1956). A protein determination, using the biuret method, was done on the suspension of n-actomyosin not used for incubation.

RESULTS

Superprecipitation studies with n-actomyosin isolated from glycerinated cardiac muscle of control and gousiekte sheep

In Fig. 1 the change in optical density as a result of superprecipitation is illustrated for gousiekte and control sheep of Group II.

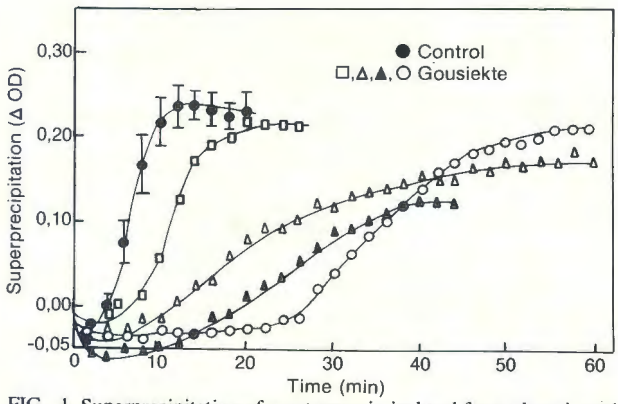

FIG. 1 Superprecipitation of n-actomyosin isolated from glycerinated cardiac muscle of control sheep(●) and gousiekte sheep with heart failure (□,Δ,▲,○)

The superprecipitation curve for controls represents the mean ± standard deviation of 4 different experiments, while the superprecipitation experiments for gousiekte sheep is shown individually. Each experiment for both gousiekte and controls was done with mixed n-actomyosin obtained from 2 different sheep.

From the data in Table 1 it can be seen that gousiekte (cardiac failure) resulted in a prolonged clearing phase as well as a decrease in the rate of superprecipitation. Although a slight non-significant decrease in the mean value is noted for gousiekte with respect to the extent of superprecipitation and clearing (increased clearing), a striking decrease is seen for some of the individual cases.

TABLE 1 Superprecipitation of n-actomyosin isolated from glycerinated cardiac muscle of control and gousiekte sheep

Parameter of superprecipitation	Control (n=4×2)	Gousiekte (n=4×2)	Significance of difference (Wilcoxon)
Extent (max. ΔOD)	0,24±0,02	0,18±0,02	P<0,10 NS
Rate (t ½; time in min at ½ max. ΔOD)	3,75±0,72	12,75±3,06	P<0,02 *
Clearing phase (min)	4,5±0,50	16,8±4,20	P<0,02 *
Extent of clearance (−ΔOD)	−0,03±0,013	−0,04±0,005	P>0,10 NS

n=No. of animals studied
* Significant difference
NS=Difference not significant

ATP-ase activity of n-actomyosin isolated from glycerinated cardiac muscle of control and gousiekte sheep

Correlation with ATP concentration

The effect of substrate (ATP) concentration on the enzyme activity of n-actomyosin from control and gousiekte sheep of Group II is shown in Table 2.

TABLE 2 Effect of substrate concentration on the enzyme activity of n-actomyosin isolated from glycerinated cardiac muscle of control and gousiekte sheep

ATP,mM	ATP-ase activity (η moles Pi/mg protein/15′)		Significance of difference (Student's t test)
	Control	Gousiekte	
0,1	413±40 (n=9)	361±17 (n=9)	P>0,10 NS
0,2	559±44 (n=9)	437±34 (n=9)	P<0,10 NS
0,4	665±52 (n=9)	461±44 (n=9)	P<0,05 *
0,6	675±59 (n=8)	452±54 (n=7)	P<0,05 *
0,8	663±60 (n=8)	437±48 (n=7)	P<0,05 *
1,0	618±44 (n=9)	407±42 (n=9)	P<0,01 *

n=No. of animals studied
* Significant difference
NS=Difference not significant

A significant depression of enzyme activity was found for gousiekte sheep at all substrate concentrations from 0,4 mM and higher. Fig. 2, which graphically represents the effect of substrate concentration on enzyme activity, shows clearly that maximum enzyme activity for gousiekte cases is reached at a lower substrate concentration; 0,4 mM ATP for gousiekte vs. 0,6 mM ATP for controls ($P < 0,05$; Student's t test). This could be indicative of an early inhibition by substrate for n-actomyosin from gousiekte hearts.

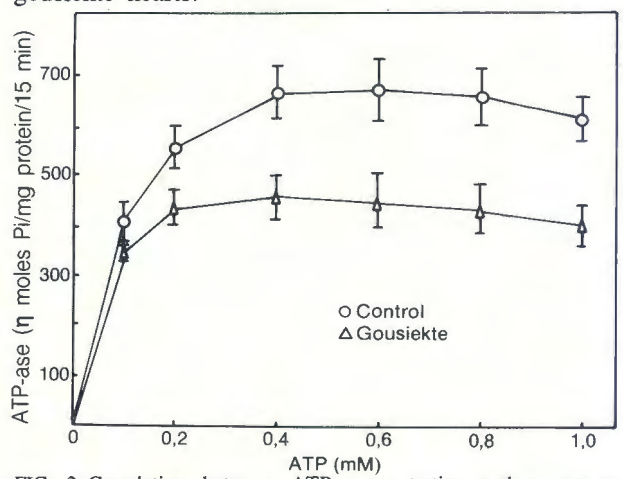

FIG. 2 Correlation between ATP concentration and n-actomyosin:ATP-ase activity of control (o-o) and gousiekte sheep (Δ-Δ)

Correlation with magnesium concentration

In Fig. 3 the ATP-ase activity of n-actomyosin isolated from glycerinated cardiac muscle of control and gousiekte sheep of Group II is correlated with an increasing concentration of magnesium.

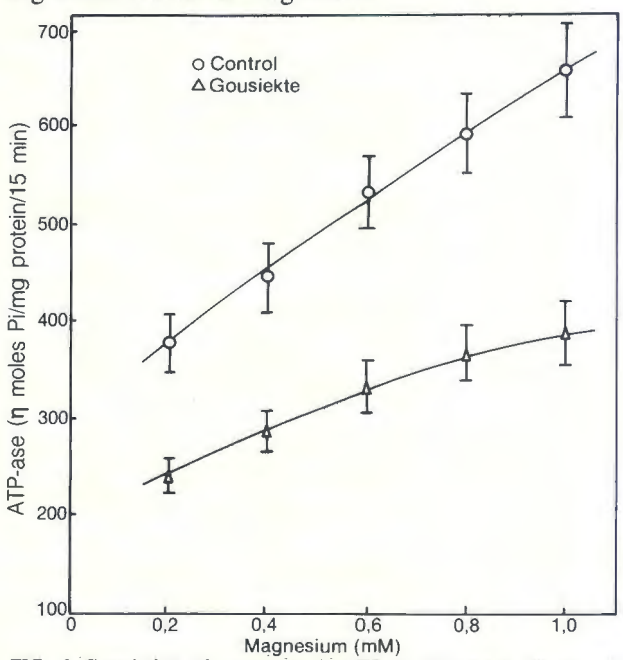

FIG. 3 Correlation of n-actomyosin:ATP-ase of control (O-O) and gousiekte (Δ-Δ) sheep, with magnesium

A significant depression in ATP-ase activity was found for gousiekte n-actomyosin at each magnesium concentration (Table 3). No significant difference in magnesium response could be demonstrated at any of the magnesium concentrations between gousiekte and control values when they were compared as the percentage increase with respect to the activity at 0,2 mM magnesium (Table 3). This finding was confirmed when tested by a growth curve analysis according to the method of Rao (1958).

TABLE 3 Effect of an increasing concentration of Mg^{2+} on (i) ATP-ase activity of n-actomyosin and (ii) percentage increase in ATP-ase activity of n-actomyosin with respect to value at 0,2 mM for control and gousiekte hearts

Magnesium (mM)	Control	Gousiekte	Significance of difference (Student's t test)	
Analysed with respect to the ATP-ase value				
	n-actomyosin:ATP-ase (η moles Pi/mg protein/15′)			
0,2	377±30 (n=9)	241±17 (n=11)	P<0,01	*
0,4	446±37 (n=9)	290±21 (n=11)	P<0,01	*
0,6	535±37 (n=9)	334±29 (n=9)	P<0,01	*
0,8	594±41 (n=8)	367±29 (n=11)	P<0,01	*
1,0	658±50 (n=9)	389±33 (n=11)	P<0,01	*
Analysed with respect to percentage increase (%)				
0,2–0,4	18,1	20,5	P>0,05	NS
0,2–0,6	43,5	37,5	P>0,05	NS
0,2–0,8	59,5	51,8	P>0,05	NS
0,2–1,0	75,6	60,4	P>0,05	NS

n=No. of animals studied
* Significant difference
NS=Difference not significant

A tendency toward a decreased response at higher concentrations of magnesium, however, is observed. This phenomenon is usually seen when the magnesium concentration exceeds that of ATP, which is not the case here. It could therefore reflect a n-actomyosin:ATP-ase which is more sensitive during gousiekte for this type of magnesium inhibition.

Correlation with calcium concentration

In Table 4 the effect of different concentrations of calcium upon (i) the ATP-ase activity and (ii) the percentage increase of the ATP-ase activity with respect to the activity at $[Ca^{2+}]=0$, is shown for n-actomyosin isolated from glycerinated cardiac muscle of control and gousiekte sheep of Group II.

TABLE 4 Effect of $[Ca^{2+}]$ on (i) ATP-ase activity and (ii) percentage increase in ATP-ase activity of n-actomyosin from control and gousiekte hearts

Calsium concentration	Control	Gousiekte	Significance of difference (Student's t test)	
Analysed with respect to the ATP-ase value				
n-actomyosin ATP-ase (η moles Pi/mg protein/15′)				
$[Ca^{2+}]=0$	209±15 (n=8)	147±7 (n=11)	P<0,01	*
pCa = 6,5	937±71 (n=7)	541±26 (n=11)	P<0,01	*
pCa = 5,2	940±96 (n=6)	602±49 (n=11)	P<0,05	*
Analysed with respect to percentage increase (%)				
$[Ca^{2+}]=0$–pCa=6,5	346	269	P<0,01	*
$[Ca^{2+}]=0$–pCa=5,2	346	309	P>0,05	NS

n=No. of animals studied
* Significant difference
NS=Difference not significant

A marked depression was noted for gousiekte n-actomyosin:ATP-ase at all concentrations of calcium, as shown in Table 4. The difference at zero calcium indicates that the differences observed at pCa=6,5 and 5,2 must in part be due to a calcium independent factor. It is, however, also attributable to a difference in calcium response as is indicated by a growth curve analysis (Rao, 1958 with Student's t test; $P < 0,01$), based on the percentage increase with respect to the ATP-ase activity at zero calcium. An analysis of the percentage increase at each of the calcium concentrations showed individually a significant higher increase for controls at pCa=6,5, but not at pCa=5,20 (Table 4). This may be the result of the higher response found for control n-actomyosin which has already reached its maximum activity at pCa=6,5 compared with the lowered response of gousiekte actomyosin which still rises to a maximum between the values pCa=6,5 to 5,20.

The sensitivity to calcium of isometric tension development by glycerinated muscle fibres from gousiekte and control hearts

To investigate the calcium sensitivity of the contractile proteins in terms of a mechanical parameter, the isometric tension development of glycerinated cardiac muscle fibres was investigated at different concentrations of calcium and expressed as a percentage of the maximum isometric tension development.

This was done for glycerinated muscle fibres of gousiekte sheep with cardiac failure (Group I) as well as for a group of sheep which received dried toxic material for a period of 11–14 days (Group III). The results are illustrated in Fig. 4 & 5. Fig. 4 indicates that a better percentage response to calcium seems to be possible for the glycerinated muscle of sheep after 11–14 days of intoxication with dried *Pachystigma pygmaeum*. This observation is supported by the fact that a statistically significant ($P < 0,05$; Student's t test) higher percentage reaction to pCa=5,90 was found for the intoxicated muscle (Fig. 4). Yet no difference could be demonstrated between intoxicated and control muscle when these data were analysed by a growth curve analysis (Rao, 1958; Student's t test). A tendency toward a slight difference in response may also be possible for gousiekte sheep (Fig. 5). From these results it seems that at least the percentage response of glycerinated muscle fibres to calcium is not depressed by the intake of dried toxic material for about 14 days, or even during gousiekte. The tendency for an increased sensitivity after 14 days of toxic material intake may perhaps be interpreted as compensatory activation at that stage of the disease.

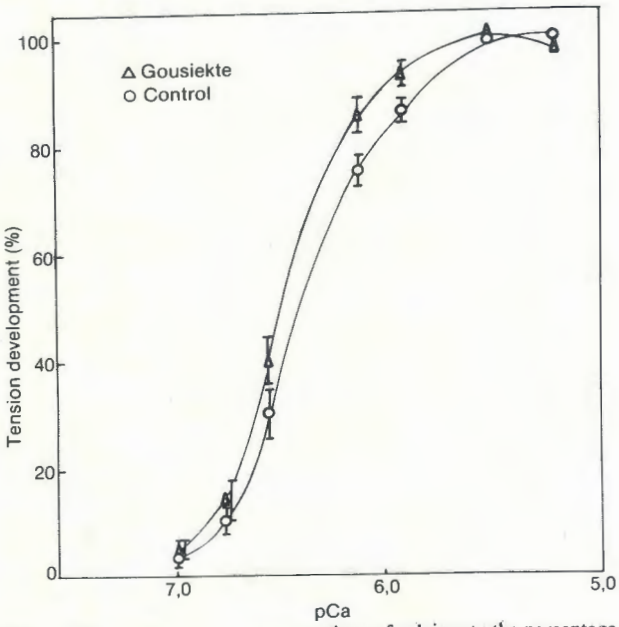

FIG 4 Effect of increasing concentrations of calcium to the percentage response of isometric tension development by glycerinated cardiac muscle strips of sheep after 11–14 days of toxic material intake (Δ–Δ) (n=4) and control sheep (o–o) (n=4)

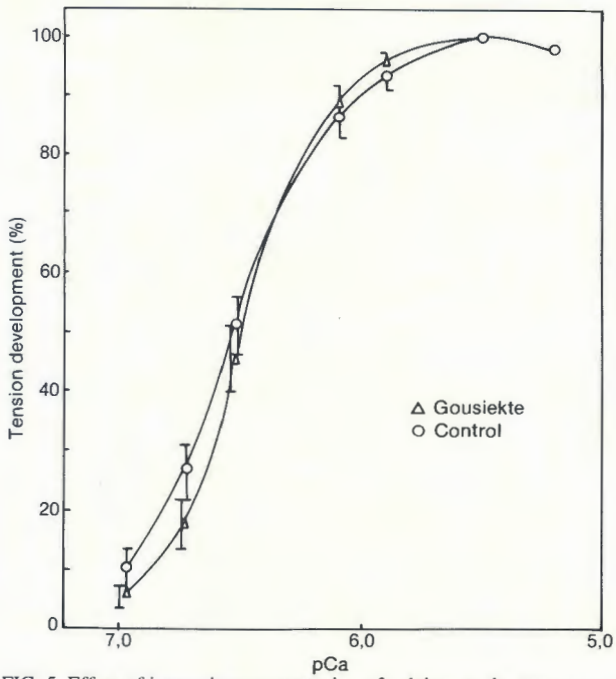

FIG 5 Effect of increasing concentration of calcium to the percentage response of isometric tension development by glycerinated cardiac muscle strips of gousiekte (Δ–Δ) (n=4) and control (o–o) (n=4) sheep

DISCUSSION

The data with respect to the properties of the contractile proteins in this report must be seen as additional to those presented in a former publication dealing with the energy production during gousiekte (Snyman *et al.*, 1982), as the samples were obtained from the same experimental animals. Clinical and post-mortem observations, which pointed to signs of congestive heart failure as well as findings of oedema of the heart muscle and absence of hypertrophy during gousiekte, thus also apply for this study.

In this study a reduced activity was found for the superprecipitation- and ATP-ase properties of gousiekte cardiac n-actomyosin; the former which is regarded as a test-tube model of muscle contraction and the latter as the biochemical manifestation of muscle contraction (Hozumi & Hotta, 1977). Ebashi (1961) stated that the increase in turbidity (during superprecipitation) surely corresponds to contraction, whereas the decrease corresponds to relaxation. The decreased rate of superprecipitation found for gousiekte cardiac n-actomyosin thus could imply a depressed contractile function.

The duration of the clearing phase, which depends on the ATP-ase activity and the concentration of ATP, is prolonged in the case of gousiekte cardiac n-actomyosin. The initial concentration of ATP, being constant for all experiments, points to a depressed ATP-ase activity for gousiekte actomyosin. This could perhaps partly be explained by some dissociation of gousiekte actomyosin as indicated by the tendency of an increased extent of clearing (not significant) by gousiekte n-actomyosin. The deduction of decreased ATP-ase is confirmed by the finding of reduced ATP-ase activity for gousiekte n-actomyosin under all conditions studied. A slower increase in ATP-ase activity with increasing concentrations of ATP for gousiekte n-actomyosin may be due to an easier inhibition by higher concentrations of substrate as a result of actomyosin dissociation.

The reduced response of the magnesium-activated cardiac n-actomyosin:ATP-ase of gousiekte cases to calcium ions is not reflected by the calcium-isometric tension response of the contractile proteins, as this was found to be about similar for gousiekte and control sheep. This finding is similar to that of Henry *et al.* (1972) for glycerinated myofibrils of hypertrophied rabbit hearts. The depressed response to calcium ions may be related to some other properties of muscle contraction such as shortening velocity (Katz, 1970).

The depressed ATP-ase activity found for the contractile system of cardiac muscle from gousiekte sheep is essentially in agreement with observations made on other types of heart failure (Alpert & Gordon, 1962; Chandler, Sonnenblick, Spann & Pool, 1967; Gordon & Brown, 1966). However, some differences with respect to the mechanisms of the deviations seem to exist. While Chandler *et al.* (1967) could not attribute the depressed myofibrillar:ATP-ase activity of failing hearts to differences in the sensitivity of the preparations to calcium and magnesium ions, Alpert & Gordon (1962) reported that the degree of response to magnesium was significantly lower for myofibrillar:ATP-ase of failing hearts. In the case of gousiekte hearts, the depressed activity of n-actomyosin:ATP-ase, with the tendency of magnesium inhibition in mind, can be attributed to a reduced sensitivity to the activating effect of calcium ions as well as to a pronounced substrate inhibitory effect. A similar result for the activating effect of calcium was found by Swynghedauw, Bouveret, Durand, Hatt, Lemaire & Piguet (1971) for myofibrils isolated from dilated hypertrophic hearts of rabbits which were affected by an experimentally induced chronic aortic insufficiency. Alpert & Gordon (1962) found no difference of substrate inhibition between myofibrils from failing and normal hearts.

These results obtained *in vitro* offer an explanation from the viewpoint of the contractile proteins for the abnormal function of gousiekte hearts. This, however, may not be the only cause, as other *in vivo* situated systems such as the energy production system may also contribute to a defective heart function during the late stages of gousiekte. The indications of an increased anaerobic energy metabolism during the late stages of gousiekte (Snyman *et al.*, 1982), for instance, leads to an accumulation of lactic acid which could result in an interference with the binding of calcium on troponin (Katz & Hecht, 1969). Furthermore, the reduced ability for accumulation of calcium ions which was reported for microsomes isolated from gousiekte hearts (Pretorius *et al.*, 1973) may also contribute to a severe disturbance of the *in vivo* regulation of contractile protein function. The extremely dilated appearance seen for most gousiekte hearts during congestive failure, which may be related to the early dissociation of n-actomyosin by ATP, may also be a factor influencing the effectivity of contractile protein function. It seems logical that this condition could be associated with a sarcomere lengthening, such that the contractile elements may function on the descending limb of the Starling curve, which will result in a reduced number of interacting sites. This means that less tension will be generated by the actin-myosin interaction, while the myosin:ATP-ase will be less activated by actin, resulting in a decreased velocity of contraction (Katz, 1970). This anatomical change, however, is believed to be the result of more fundamental changes, such as the contractile proteins itself.

When the recent data on the production and utilization of energy by the heart muscle of gousiekte sheep are considered, it seems to be a case of a reduced capability for the production of energy (Snyman *et al.*, 1982) concomitant with a reduction in the ability of energy utilization by the contractile proteins. This, integrated with the physiological state of the heart during gousiekte (e.g. a decline in the efficiency of the heart's pumping mechanism as a result of dilatation leading to a state of tachycardia which will result in an increased demand for energy), could cause the depletion of the energy stores.

Whether the deviations in the properties of the contractile proteins is a primary lesion as a result of gousiekte-bossie intoxication is not clear at this stage and needs further investigation.

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