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A STUDY ON THE FUNCTION OF SOME SUBCELLULAR SYSTEMS OF THE SHEEP MYOCARDIUM DURING GOUSIEKTE. I. THE ENERGY PRODUCTION SYSTEM*

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ABSTRACT

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In order to determine the status of the energy production system of the heart during cardiac failure of sheep with gousiekte, observations were made of the heart tissue levels of adenosine triphosphate (ATP), creatine phosphate (CrP), inorganic phosphate, reduced nicotine adenine dinucleotide (NADH) and lactate. Some measurements on oxidative phosphorylation were also made. A significant decrease in ATP and CrP levels coincided with a simultaneous rise in the ATP:CrP ratio and lactate levels in gousiekte hearts. No significant deviations in inorganic phosphate and NADH levels could be demonstrated. These abnormalities were accompanied by a decreased uptake of oxygen by isolated mitochondria of gousiekte hearts. There was a marked increase in the anaerobic state of the hearts of dying gousiekte sheep, while the values of NADH and the ATP:CrP ratio at a presymptomatic stage indicated a possible early derangement in the energy metabolism of sheep fed the toxic material. No hypertrophy could be detected for the failing ventricles of gousiekte sheep after being corrected for a significant amount of oedema found in the heart tissue of these animals.

It was concluded that the depressed ATP and CrP levels in the heart tissue of gousiekte sheep during cardiac failure could at least in part, be attributed to a depressed aerobic energy production. It is not possible, however, to state whether this is a primary or a secondary response due to intoxication and also whether it could be seen as a cause or effect of cardiac failure.

INTRODUCTION

Gousiekte**, classified as a toxic congestive cardiomyopathy in ruminants, is characterized by a latent period of about 2–6 weeks followed by sudden death (McKinney, 1974; Pretorius & Terblanché, 1967). The disease is caused by ingestion of 5 different members of the plant family, Rubiaceae, namely: *Pachystigma pygmaeum* Schltr.) Robyns (Theiler, Du Toit & Mitchell, 1923); *Pachystigma thamnus* Robyns (Adelaar & Terblanché, 1967); *Pavetta harborii* S. Moore (Uys & Adelaar, 1957) *Pavetta schumaniana* F. Hoffm (Naudé & Adelaar, unpublished data, 1962) and *Fadogia monticola* Robyns (Hurter, Naudé, Adelaar, Smit & Codd, 1972).

Post-mortem analysis of gousiekte cases reveals congestion and oedema of the lungs, dilatation of the heart and varying degrees of hydrothorax, ascites, hydropericardium and some other minor abnormalities (Theiler *et al.*, 1923; Steyn, 1949; Adelaar, Terblanché & Smit, 1966). At autopsy, the heart is found not to be in *rigor mortis*. According to McKinney's (1974) interpretation, the muscle fibres are so involved by the toxic process that they are unable to respond to any stimuli, even that of death.

The clinical symptoms and haemodynamic characteristics during both the latent period and the acute phase of the disease are well documented (Pretorius & Terblanché, 1967; Pretorius, Terblanché, Van der Walt & Van Ryssen, 1973). Definite symptoms of cardiac failure such as systolic murmurs, gallop rhythm, QRS amplitude alternation and bundle branch block were found. An early decrease in myocardial contractility was indicated by conspicuous changes in the ultra low frequency acceleration ballistocardiogram and aorta blood flow recordings (Pretorius *et al.*, 1973). It was furthermore noticed that unifocal ectopic beats in the affected animals readily changed to multifocal premature beats after exercise (Pretorius & Terblanché, 1967). These findings might suggest a relative state of ischaemia of the cardiac tissue during gousiekte as part of a more general derangement of the energy transformation mechanisms of the myocardium (Pretorius *et al.*, 1973). This suggestion is in accordance with the postulation that there exist at least 2 molecular classes of heart failure, namely, that in which the defect lies in the generation of ATP and that in which the defect lies in the utilization of ATP (Olson, 1956; Pool, Spann, Buccino, Sonnenblick & Braunwald, 1967). Since the status of high-energy phosphate in the heart reflects a balance between production and utilization of energy, it was decided to first investigate the possibility of such an imbalance by determining the levels of ATP and CrP. Having demonstrated such an imbalance, other parameters of the energy metabolism in relation to energy production were detected in an attempt to explain the nature and cause of the deviation.

MATERIALS AND METHODS

Induction of experimental gousiekte

Merino wethers (2–4 teeth) from an area free of gousiekte were used for this study. On arrival, the sheep were immunized against bluetongue, pulpy kidney and pasteurellosis and dosed against internal parasites with thiabendazole. The animals were fistulated (rumen) and put on a daily ration consisting of 200 g mealie meal plus lucerne hay *ad lib*. They were also supplied with a salt-bone meal (1:1) lick and water *ad lib*. After a recovery period of about 2 weeks feed and water was withheld overnight and the sheep dosed with *Pachystigma pygmaeum* each morning.

The *Pachystigma pygmaeum* was collected twice a week from the Swartrand area in the Koster district from abouth the middle of December to the end of the growing season before the first frost. It was usually collected in the forenoon and then transported in jute bags within 2 hours to the laboratory. There it was stored at 6 °C and used up in about 3–4 days. Before being administered to the sheep, the plants were washed with water to clean them from sand and cut into 2 cm pieces with scissors.

On arrival at the laboratory, some of the plants were dried in a room at about 40 °C, whereafter they were also stored at 6 °C and broken by hand shortly before being dosed.

The sheep were dosed through the rumen fistula every morning at a rate of 25 g wet material/kg body mass/day. This was shown to be a lethal dose, if fed for a period of

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** The word gousiekte, literally translated from the Afrikaans, means "quick disease", referring to the sudden onset of death

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8 days (T. W. Naudé, unpublished data, 1967). Dosage in this experiment, however, continued until gousiekte was induced.

Clinical observations were conducted daily on these animals for diagnosing gousiekte. Gousiekte was diagnosed when one or more of the following symptoms of heart failure consistently occurred; tachycardia (>100), a gallop rhythm and a systolic murmur (Pretorius & Terblanché, 1967). Body temperature was also taken for any indications of a possible infection. When gousiekte was diagnosed, the sheep was killed by rapid cardiectomy and samples of the heart muscle were taken for analysis as described later on.

Gousiekte was induced in 3 different groups of sheep over a period of 4 years, namely, Group I (1973, 1974), Group II (1975) and Group III (1976). The sheep of yet another group, Group IV (1974), received 200–300 g of the dried *Pachystigma pygmaeum* for 11–14 days and were then killed without showing any prominent symptoms of gousiekte.* Control sheep in each group were similarly treated as gousiekte sheep, except that they were not fistulated and not intoxicated with *Pachystigma pygmaeum*. Sampling from controls was taken equally with gousiekte cases.

All sheep were penned individually in well-ventilated stalls.

Sampling

All control and experimental animals were anaesthetized with sodium pentobarbital while being artificially respirated with oxygen, and were killed by rapid cardiectomy. Before the heart was removed, a portion of the beating apex was seized with tongs precooled in liquid nitrogen (Wollenberger, Ristau & Schotta, 1960). The sample was then powdered in a mortar under liquid nitrogen, transferred to a small holder, stoppered, and stored in liquid nitrogen until being analysed for ATP, CrP, inorganic phosphate, lactic acid and NADH.

A portion of the left ventricle, free of gross fat, was cut out immediately after the heart was removed, and minced into a 4 °C KEA-medium (0,18 M KC1, 10 mM EDTA, 0,5% Albumin) for the isolation of mitochondria. Another small portion was cut out, its mass determined, and dried at 110 °C for 24 h to determine the water content. The mass of the rest of the ventricles and the carcass of the sheep was also determined for estimating hypertrophy (the mass of the pieces cut off was added to the ventricle mass.)

Biochemical analysis

For the determination of ATP, CrP and inorganic phosphate, the powdered tissue was extracted with 0,3 M perchloric acid at 0 °C. After centrifugation, part of the supernatant was used to determine the CrP (Furchgott & De Gubareff, 1956) and inorganic phospate by the phosphomolibdate method of Fiske & Subbarow (1925), as modified by Furchgott & De Gubareff (1956).

Another part of the supernatant was diluted 10 times with water to determine ATP by the firefly luminescence method of Strehler & McElroy (1957). An aliquot (0,2 mℓ) of the diluted supernatant was rapidly and reproducibly blown out of a microlitre pipette into 1 mℓ of firefly lantern extract in a quartz cuvette at 20 °C. The intensity of light emission was recorded with a Beckmann model D. U. spectrophotometer. Peak luminescence was related to the concentration of ATP.

NADH was determined enzymatically by the method of Klingenberg (1963). Four to 6 determinations were done on each sample.

Lactate in the heart muscle was determined enzymatically according to the Sigma Technical Bulletin No. 826. The extract used for this determination was made at 4 °C, using 1 mℓ of perchloric acid and 100–250 mg of powdered muscle tissue.

For oxidative phosphorylation studies, the minced tissue was homogenized with an Ultraturrax in 12 volumes of KEA-medium and the mitochondria isolated, as described by Sordahl, Johnson, Blailock & Schwartz (1971). The isolated mitochondria were suspended in a fortified incubation medium as used by Lochner, Opie, Owen, Kotzé, Bruyneel & Gevers (1975), and the oxygen uptake determined manometrically (Lochner, Opie, Brink & Bosman, 1968), using a Gilson Respirometer. Phosphorylation during the same time interval was calculated from the inorganic phoshate which had not been taken up. The inorganic phosphate was determined as described, while mitochondrial protein was determined by the biuret method (Garnall, Bardawill & David, 1949).

Results

Clinical findings

Tachycardia and/or a gallop rhythm were used as criteria for diagnosing heart failure in gousiekte sheep. A systolic murmur was also considered as an additional indication of heart failure. According to these criteria, all the gousiekte sheep of Groups I, II and III revealed symptoms of heart failure at autopsy.

A post-mortem analysis showed signs of peritoneal and pleural effusions in many cases. An abnormally enlarged (dilated) heart *in vivo* with pronounced hydropericardium was seen in most cases of gousiekte, especially in Groups II and III.

The gousiekte sheep in Group IV did not show any signs of heart failure during auscultation and post-mortem analysis, except in 1 case where the heart frequency exceeded a hundred.

An analysis of the water content of the ventricle muscle indicated a significant increase for gousiekte sheep ($P<0,01$) during cardiac failure (Table 1). No such increase, however, could be detected for the sheep of Group IV ($P>0,05$) after 11–14 days of the intake of toxic material (Table 4). A significant increase in ventricle mass ($P<0,01$) and ventricle mass:carcass mass ($P<0,01$) was also detected for gousiekte sheep during cardiac failure (Table 1). However, when these parameters were corrected for normal water content, there was no significant difference ($P>0,05$) in ventricle mass between gousiekte and control sheep (Table 1). The carcass masses for control and gousiekte sheep were the same ($P>0,05$) and thus could not have influenced the parameters of hypertrophy.

Changes in ATP and CrP

A significant reduction of ATP and CrP was observed during cardiac failure, when either the individual groups ($P<0,01$) or the total number of gousiekte sheep ($P<0,01$) were compared with the controls (Table 2). This depression of high-energy phosphate content in heart muscle tissue was also seen for dying gousiekte sheep* (Table 3). However, the values for CrP were significantly lower than the values of non-dying gousiekte sheep ($P<0,05$), whereas the ATP values did not differ significantly ($P>0,05$). No significant difference in ATP and CrP content could be demonstrated for sheep which were fed the toxic material for a period of only 11–14 days ($P>0,05$) (Table 4).

* It takes about 30 days and more to induce gousiekte with fresh (wet) material

* Gousiekte sheep which were dying during cardiectomy, most probably as a result of heart failure. Samples were taken only when the heart was still showing some physical activity

TABLE 1 Parameters of hypertrophy and oedema for ventricles from control and gousiekte sheep

Group No.	Dry/wet mass (%) (ventricle tissue)	Ventricle mass (g)	Ventricle mass corrected for normal water content (g)	Ventricle/carcass mass (%)	Ventricle/carcass mass corrected for normal water content	Carcass mass (kg)
			Control			
II (5)†	20,9±0,2	86,6±3,8	86,6±3,8	0,55±0,03	0,55±0,03	15,7±0,6
III (10)	20,5±0,1	88,7±4,9	88,7±4,9	0,55±0,02	0,55±0,02	16,1±0,8
			Gousiekte			
II (6)	18,2±0,4	101,9±2,4	89,5±3,0	0,66±0,02	0,58±0,02	15,3±0,5
III (7)	17,7±0,2	106±5,8	90,5±4,8	0,60±0,03	0,51±0,03	17,8±0,9
	*	*	NS	*	NS	NS

*=P<0,01 for significance of difference (analysis of variance and a multiple comparison, Student Newman Keuls)
NS=Not significant (P>0,05)
† Number in parentheses indicates number of animals used

TABLE 2 ATP and CrP content of the heart muscle of control and gousiekte sheep

Group No.	No. of animals	ATP (μ moles/g wet mass)	CrP (μ moles/g wet mass)	ATP:CrP
		Control		
I	7	5,60±0,49	11,33±1,10	0,51±0,05
II	5	5,94±0,09	11,48±0,25	0,52±0,01
III	13	5,91±0,12	10,92±0,27	0,54±0,01
		Gousiekte		
I	8	3,58±0,24*	5,86±0,81*	0,76±0,19
II	6	4,50±0,16*	7,35±0,56*	0,63±0,05
III	8	4,79±0,29*	7,10±0,49*	0,68±0,03*
		**	**	**

*=P values of 0,05 or less for significance of difference (Student's t test) for each gousiekte group from the corresponding control group
**=P values of 0,05 or less for significance of difference (analysis of variance) of total gousiekte sheep from total control sheep

TABLE 3 Heart tissue levels of metabolites of energy metabolism for gousiekte sheep in the stage of dying

Sheep No.	ATP (μ moles/g)	CrP (μ moles/g)	ATP/CrP	NADH (η moles/g)	Lactate (μ moles/g)	Inorganic phosphate (μ moles/g)
1 (I)†	2,8	2,4	1,16	—	—	—
2 (II)	4,7	4,6	1,02	289	3,0	4,05
3 (II)	4,6	5,9	0,78	—	—	4,70
4 (III)	4,7	1,3	3,6	348	10,4	6,80
	4,2±0,47	3,6±1,04	1,64±0,66	318±30	6,7±3,7	5,18±0,83
	NS	*	*			

† Number in parentheses indicates the group to which the sheep belong
*=P values of 0,05 or less for significance of difference (two-sample Wilcoxon test) for dying gousiekte sheep compared with total gousiekte sheep

TABLE 4 Heart tissue levels of ATP, CrP, NADH and ATP:CrP ratio, and dry mass:wet mass for ventricle tissue, of control sheep and sheep after 11–14 days of toxic material intake

Sheep	ATP (μ moles/g)	CrP (μ moles/g)	ATP:CrP	NADH (η moles/g)	Dry mass:wet mass (ventricle tissue)
Control (4)†	5,6±0,4	10,9±0,77	0,51±0,33	138±9,5	20,8±0,4
Gousiekte (4)	6,4±0,8	10,3±1,5	0,61±0,01	63±14,7	22,0±0,8
	NS	NS	*	*	NS

*=P<0,02 for significance of difference (two-sample Wilcoxon test) of gousiekte from control
NS=Not significant
† Number in parentheses indicates number of animals studied

TABLE 5 Heart tissue levels of NADH, Lactate and inorganic phosphate of control and gousiekte sheep

Group No.	NADH (η moles/g wet mass)	Lactate (μ moles/g wet mass)	Inorganic phosphate (μ moles/g wet mass)
		Control	
II	102,2±20,3 (4)	1,80±0,38 (5)	2,34±0,27 (5)
III	129,9±7,4 (11)	1,36±0,10 (13)	2,85±0,19 (13)
		Gousiekte	
II	118,0±7,6 (5)	2,30±0,56 (6)	2,40±0,22 (6)
III	145,5±22,3 (8)	3,09±0,61 * (7)	3,25±0,31 (8)
	NS	**	NS

*=P values of 0,05 or less, for significance of difference (Student's t test) of each gousiekte group from the corresponding control group
**=P values of 0,05 or less, for significance of difference (analysis of variance) of total gousiekte sheep from the total control sheep

TABLE 6 Oxidative phosphorylation of mitochondria isolated from the hearts of gousiekte and control sheep of Group III (α-ketoglutaric acid as substrate)

Sheep	Oxygen uptake (μ atoms/mg protein per 20 min)	Uptake of inorganic phosphate (μ moles/mg protein per 20 min)	P/O
Control (7)†	2,27±0,16	4,73±0,49	2,07±0,10
Gousiekte (5)	1,73±0,09 *	3,92±0,30 NS	2,26±0,11 NS

*=P<0,05 for significance of difference (two-sample Wilcoxon test) of gousiekte from control
NS=Not significant
† Number in parenthesis indicates number of animals used

The decrease (%) in CrP exceeded that for ATP, as is indicated by the rise in the ATP:CrP ratio (P<0,05) (Table 2). Once again a significantly higher ratio for dying gousiekte sheep was found compared to that of non-dying gousiekte sheep (P<0,05) (Table 3). This high ratio of ATP:CrP reflects the very low values of CrP, since ATP values were similar to those of non-dying gousiekte sheep. A small rise in the ATP:CrP ratio (P<0,02) was also noted for gousiekte sheep after 11–14 days of the intake of toxic material (Table 4), in this case due mainly to a small non-significant rise in ATP.

The correlation between the tissue content of ATP and CrP is illustrated in Fig. 1. By fitting a linear regression-line through the values of the sheep in Groups II and III, the much higher depression (%) of CrP values compared to those of ATP can clearly be seen. For example, an ATP value of 6 corresponds to an ATP:CrP value of 0,54, while an ATP value of 4 corresponds to an ATP:CrP value of 0,80. It may be of significance to note that only one point lies below an ATP value of 4,1, including the values for dying gousiekte sheep which were not taken into consideration when the regression line was fitted.

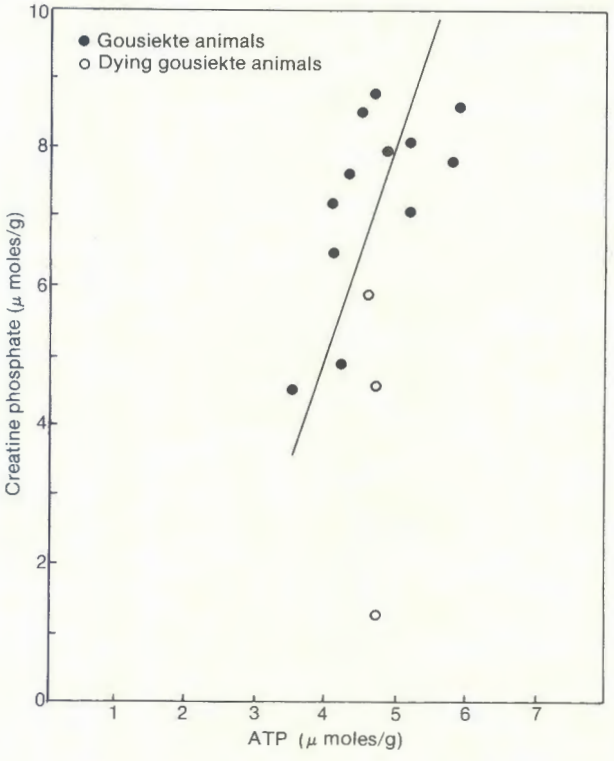

FIG. 1 Relation between the ATP- and creatine phosphate content of the heart muscle from gousiekte sheep

Lactic acid, inorganic phosphate and NADH

Table 5 shows the values of NADH, lactate and inorganic phosphate for control and gousiekte sheep. No difference was found for NADH and inorganic phosphate, while a significant rise in lactate (P<0,01) was found for gousiekte sheep. Abnormally high values for NADH were seen for individual gousiekte sheep in the state of dying (Table 3). Lactate values for these sheep were equal to and far above those for non-dying gousiekte sheep, while all the values for inorganic phosphate were also above the mean value for non-dying gousiekte sheep (Table 3). On the contrary, after 11–14 days of the intake of toxic material (dried) a significant lower content of NADH (P<0,02) was found, when compared with that of controls (Table 4).

Mitochondrial oxidative phosphorylation

Table 6 shows some parameters with respect to the mitochondrial function of heart tissue. No difference could be detected for the P/O ratio and inorganic phosphate uptake between gousiekte and controls with α-ketoglutarate as substrate. A depressed capacity for electron transport as indicated by a lowered oxygen uptake (P<0,05) was found, however.

DISCUSSION

In this study, anatomical evidence of congestive heart failure with no hypertrophy was found for gousiekte sheep. These findings are in agreement with the observations made by Pretorius *et al.* (1973). A significant degree of oedema of the heart muscle was also detected. According to histological studies by Jacobson presented by McKinney (1974), this is probably located in the interstitial spaces.

Pretorius & Terblanché (1967) reported that a relative state of ischaemia may be present in the hearts of gousiekte sheep, probably as part of a more general derangement of the energy transformation mechanisms of the myocardium. The increase in lactate which was found in the hearts of gousiekte sheep may support this suggestion. It is also corroborated by the faster and more marked reduction of CrP content than of ATP, an observation which was also made in other types of heart failure (Feinstein, 1962; Fox, Wikler & Reed, 1965; Lochner, Brink & Van der Walt, 1970; Pool & Seagren, 1966; Pool *et al.*, 1967) reflecting a shift to anaerobic metabolism. However, the fact that no significant rise in NADH and inorganic phosphate levels could be demonstrated for the hearts of gousiekte sheep may be an indication of an anaerobic situation which is to some extent still under control. It should be kept in mind, however, that the possibility of an outward diffusion of inorganic phospate and lactate (Mathur & Case, 1973) might prevent the build-up of extremely high levels of these metabolites in heart muscle and may therefore complicate the quantitative interpretation of these values.

The increased anaerobic state is related to depressed levels of ATP and CrP and may be indicative of an inadequate aerobic energy production. The depressed oxygen uptake noted for isolated mitochondria could offer some explanation for the shift to anaerobic metabolism

and the depressed contents of ATP and CrP. No uncoupling of oxidative phosphorylation could be detected. Experimental factors such as the composition of isolation or incubation medium, however, may influence the results obtained by *in vitro* experiments (Lochner *et al.*, 1975). It should thus be interpreted with caution. The defect in oxygen uptake, however, is in agreement with the statement of Schwartz, Sordahl, Entman, Allen, Reddy, Goldstein, Luchi & Wyborni (1973), that a defect in mitochondrial energy production is associated with severe heart failure. Although a direct effect cannot be excluded, the mitochondrial damage may be a result of mechanical distortion caused by congestive failure (Opie, 1969). The influence of some *in vivo* factors during heart failure may also contribute to a defective mitochondrial energy production. It was speculated that defects in the function of sarcolemma and sarcoplasmic reticulum may lead to intramitochondrial accumulation of calcium (Dhalla, 1976) leading to uncoupling of oxidative phosphorylation (Lehninger, 1949). The depressed uptake of calcium ions by isolated fragments of sarcoplasmic reticulum noted in gousiekte hearts (Pretorius, *et al.*, 1973) may thus indirectly contribute to the depressed levels of ATP and CrP. The uncoupling effect of free fatty acids (Borst, Loos, Christ & Slater, 1962; Hüllsmann, Elliott & Slater, 1960) is another factor to consider for the *in vivo* situation.

Data obtained for dying gousiekte sheep, which were presumably in a final state of cardiac failure, reflected a drastic increase in metabolites occurring during an anaerobic state. This could in part be attributed to a relative state of ischaemia due to a decrease in coronary blood flow. It is important to note that only 1 of these sheep had an ATP content lower than 4,1μ moles/g of heart tissue, which clearly shows that the cause of there death was not due to ATP depletion, for the heart muscle can apparently survive and maintain contraction at ATP levels as low as 1,5 to 2,0 μ moles/g of tissue (Gudbjarnason, Mathes & Ravens, 1970). Considering the high anaerobic state of these hearts and consequently the elevated levels of lactic acid, the cessation of contraction in the presence of apparently adequate stores of ATP could be explained by one or both of the following hypotheses: the rise in hydrogen ion concentration could interfere with the calcium binding sites of the excitation contraction coupling process (Katz & Hecht, 1969) and/or an inhibition of intracellular energy transfer which may lead to a depletion of extra-mitochondrial energy stores (Gudbjarnason *et al.*, 1970) and thereby reduce the availability of ATP for the contractile process.

It is not possible to state whether these deviations of the energy metabolism in the hearts of gousiekte sheep are primary or secondary in the genesis of heart failure, since abnormalities in the function of sarcoplasmic reticulum has also been reported for the hearts of gousiekte sheep (Pretorius *et al.*, 1973), while other subcellular functions like those of the sarcolemma and ribosomes have not been reported on yet. It may be that these deviations in energy metabolism in the hearts of gousiekte sheep could be a final common path for many types of damage caused in different ways, since a depression in the levels of ATP and CrP have been found for various types of heart failure (Fedelesova & Dhalla, 1971; Feinstein, 1962; Fox *et al.*, 1965; Pool *et al.*, 1967; Lochner *et al.*, 1970; Skinner, Scott, Morrison, Imai, Jarmolych & Lee, 1973). Schwartz *et al.*, (1973) concluded that severe heart failure is characterized by defects in mitochondrial energy production. This, however, does not necessarily exclude the possibility of a causative role for the energy metabolism in the genesis of heart failure during gousiekte. A tendency for a lowered NADH associated with a rise in the ATP:CrP ratio in the heart muscle was noticed in this study for sheep after only 11–14 days* of the intake of toxic material, without any signs of heart failure. This finding could probably be related to the early decrease of myocardial contractility as indicated by conspicuous changes in the ultra-low frequency acceleration ballistocardiogram and aorta blood flow recordings which were noted (Pretorius & Terblanché, 1967), or may be due to compensatory changes.

According to the hypothesis of Olson (1956), heart failure may be caused by a defect in energy production and/or a defective mechanism for energy utilization. The depressed contents of ATP and CrP in the heart muscle of gousiekte sheep measured in this study are explained by a decrease in aerobic energy production. This does not exclude the possibility of a simultaneous contribution by a defect of the energy utilizing process, however. In view of the quantitative importance of the contractile protein system with respect to energy utilization together with the fact that defects of the ATP-ase activity of the contractile protein complex during cardiac failure have been reported (Schwartz *et al.*, 1973), it seems necessary also to investigate this system during gousiekte.

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* Normally, a period of 21–35 days of toxic material intake was necessary before any symptoms of gousiekte could be detected

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