Onderstepoort J. vet. Res., 57, 157–161 (1990)

A COMPARISON OF HAEMODYNAMIC AND VASOCONSTRICTORY RESPONSES IN SHEEP WITH A TOXIC FRACTION FROM *PACHYSTIGMA PYGMAEUM* AND WITH THE PLANT MATERIAL

J. J. VAN DER WALT[1], J. M. VAN ROOYEN[1] and A. P. LÖTTER[2]

ABSTRACT

VAN DER WALT J. J., VAN ROOYEN, J. M. & LÖTTER, A. P., 1990. A comparison of haemodynamic and vasoconstrictory responses in sheep with a toxic fraction from *Pachystigma pygmaeum* and with the plant material. *Onderstepoort Journal of Veterinary Research*, 57, 157–161 (1990)

ST-segment changes in the ECG, which are an indication of acute myocardial ischaemia, are obtained when a small quantity of an extract from dried *Pachystigma pygmaeum* is injected intravenously in sheep. When the fraction was injected subcutaneously, animals reached a crisis after about 5 h, with low values of stroke volumes and high values for pulmonary arterial pressures, pulmonary vascular resistances and heart rates. The haemodynamic changes are an indication of the development of pump failure of the heart. In sheep, injected subcutaneously with the toxic fraction, as well as for sheep dosed with plant material through rumen fistula, increased serum levels for thromboxane and increased or decreased levels for prostacycline were observed. The experimental results are interpreted as being an indication that these prostaglandines may be involved in the development of gousiekte by impeding cardiopulmonary function as a result of coronary and pulmonary vasoconstriction. The sudden death observed in some gousiekte sheep may be due to myocardial ischaemia and associated arrhythmias.

INTRODUCTION

Gousiekte is a disease which has been studied extensively for some time (Walker, 1908; Theiler, Du Toit & Mittchell, 1923), but no solution for the problem has been found. It causes large losses in domestic ruminants grazing on fields where certain plants of the family *Rubiaceae* such as *Pachystigma pygmaeum* occurs (Kellerman, Coetzer & Naude, 1988). Although the clinical symptoms and sudden death after a latency period of 4–8 weeks are indications of heart failure, the precise physiological mechanisms involved are unknown. Pathological lesions, often observed, are multifocal—diffuse myocardial fibroses with mild to moderate round cell infiltration (Theiler *et al.*, 1923) as well as various degrees of ventricular dilatation and thinning of the free ventricular walls (Prozesky, Fourie, Neser & Nel, 1988).

The most prominent clinical symptoms of gousiekte are dispnoea, tachycardia and gallop rhythms. The changes in the heart sounds are usually a splitting of the first sound and systolic murmurs, while ECG changes, which are typical of a variety of arrhythmic conditions, occur (Pretorius & Terblanche, 1967). The most prominent haemodynamic changes are increased ventricular filling pressures, decreased stroke volumes and cardiac outputs. Ventricular failure occurs when the stroke volume decreases from a control value of about 50 mℓ to 40 mℓ for left- and to 20 mℓ for right- and bi-ventricular failure (Van der Walt, Van Rooyen, Cilliers, Van Ryssen & Van Aarde, 1981). The most sensitive diagnostic haemodynamic criterion for diagnosis of gousiekte is the cardiopulmonary flow index (CPFI), which is the ratio of the cardiopulmonary blood volume to stroke volume (Van der Walt *et al.*, 1981). Early diagnosis of gousiekte is possible through the detection of increased levels of aspartate transaminase (Fourie, Schultz, Prozesky, Kellerman & Labuschagne, 1989).

The aim of this study was to investigate the mechanisms involved in gousiekte by comparing the effects of intravenous or subcutaneous administration of a toxic fraction extracted from *Pachystigma pygmaeum* with those of the terminal phase inducted by dosing sheep with the plant material through rumen fistula.

MATERIALS AND METHODS

Twenty-eight full-grown sheep were dosed either with sun-dried *Pachystigma pygmaeum* through rumen fistula or with an intravenous or subcutaneous injection of an active extract of dried plant material. The dosage through rumen fistula was 300 g/day for dried plant material. The dosing with plant material was stopped as soon as the CPFI started to rise above values of 9, which was within 21 days for 1 sheep and 34 days for the other. For intravenous and subcutaneous administration of the toxic fraction 2–10 mg and 0,05–5 g were used respectively. In 20 animals intravenous injections were given with intervals varying from 7–10 days. ST-segment changes of the ECG and pulmonary arterial pressure changes were recorded for these animals during a period of 20 min after each injection. Desensitization was observed at a later stage on a group of 5 of these animals by injecting a small dose every day or by injecting a progressively larger dose twice a week. The sensitivity of these animals for the toxic fraction were determined at regular intervals with a small intravenous injection. Two groups of 4 animals were used for recording changes in haemodynamic parameters and prostaglandines respectively.

The active extract was obtained by mixing pulverized dried leaves with calcium carbonate and keeping it in a water suspension for 24–48 h. The solution was then pressed out, filtered, and a fraction precipitated with methanol. The precipitate was dried and stored at 4 °C. Before use, the precipitate was dissolved in distilled water, centrifuged at 10 000 rpm for 1 min, and the supernatant injected.

Post mortem analysis for the 2 sheep dosed through rumen fistula were typical of congestive miocardial failure and were indentical to that obtained in another project in which 34 animals were used (Van der Walt *et al.*, 1981).

Cardiac output was measured by using the thermodilution technique with a 7 F-110 cm Swan-Ganz

[1] Department of Physiology, and

[2] Institute of Industrial Pharmacy, Potchefstroom University for CHE, Potchefstroom, Republic of South Africa

Received 15 May 1990—Editor

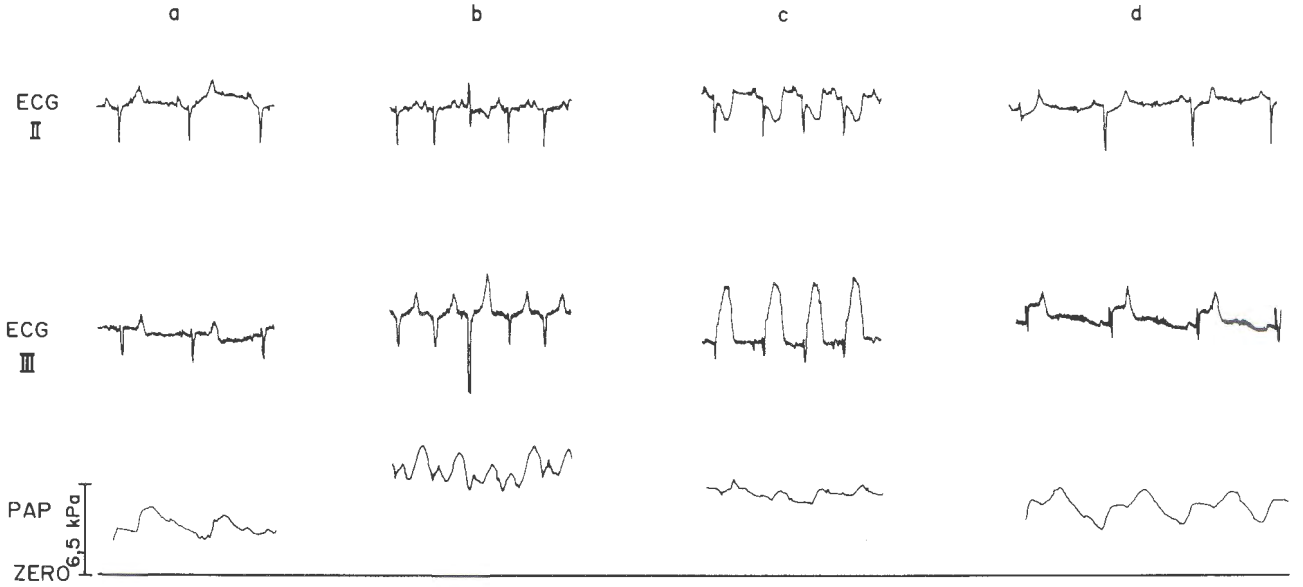

FIG. 1 Changes in ECG (leads 11 and 111) and in the pulmonary arterial pressure (PAP) taking place consecutively during the first 120 s after an intravenous injection of 5 mg of the extracted fraction, a = control, b = a vertricular extrasystoly, c = large T-waves and ST-segment shifts and d = the animal collapsed

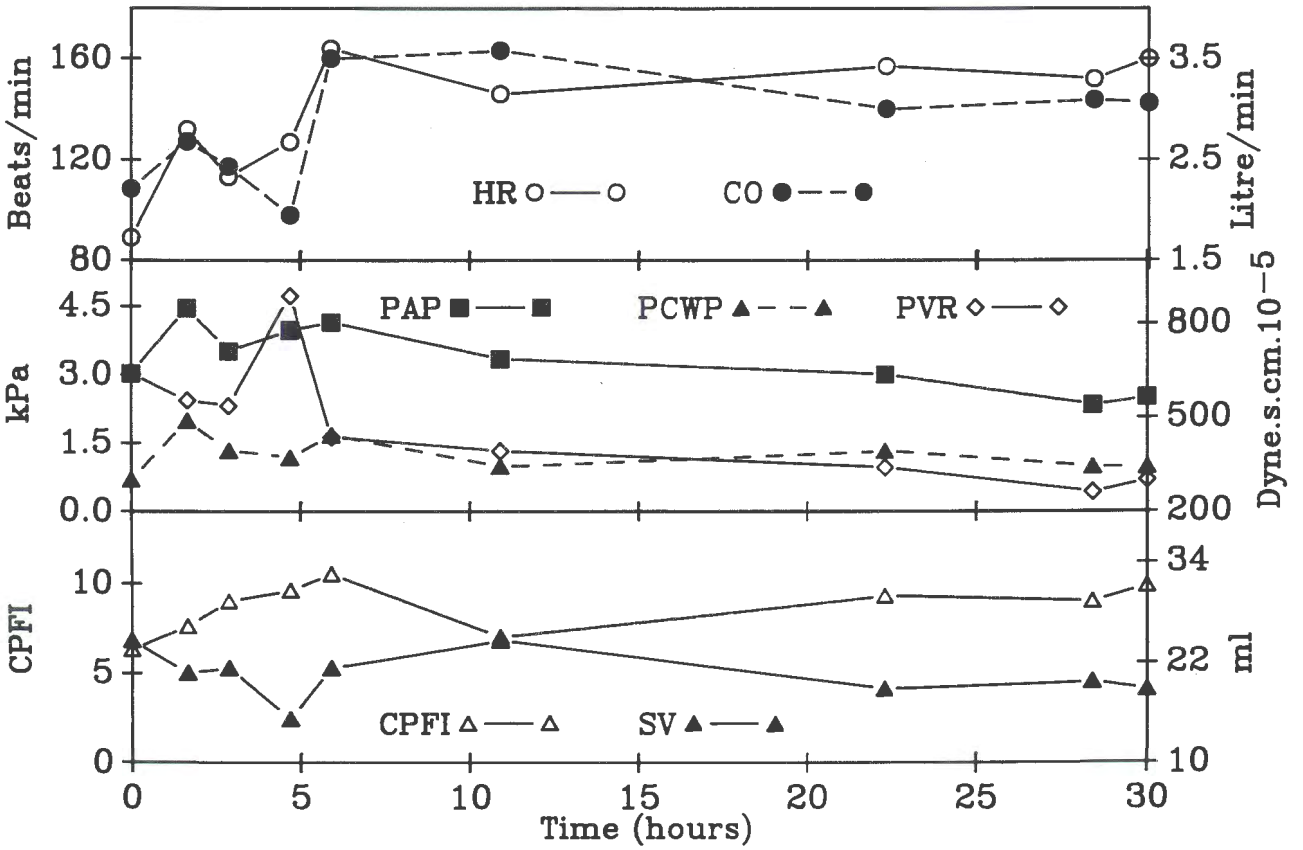

FIG. 2 Changes in haemodynamic parameters as a result of a subcutaneous injection of 2 g of the extracted fraction.
Heart rate = HR, cardiac output = CO, pulmonary arterial pressure = PAP, pulmonary capillary wedge pressure = PCWP, pulmonary vascular resistance = PVR, cardiopulmonary flow index = CPFI and stroke volume = SV

catheter and a cardiac output computer[1]. With the catheter, right atrial pressure, pulmonary arterial pressure and pulmonary capillary wedge pressure were determined. The cardiopulmonary flow index (CPFI) was determined by recording the flow of isotope through the heart and lungs with a sodium iodide crystal after injection of a bolus of technetium pertechnetate. The CPFI is calculated as quotient of the transit time of the isotope through the lungs and the period of the heartbeat. Under control conditions, the CPFI has a value of 7 but with heart failure it increases to values in excess of 14 (Van der Walt *et al.*, 1981; Van Rooyen & Van der Walt, 1989). Thromboxane and prostacycline in the serum were

[1] Edwards Model 9520A

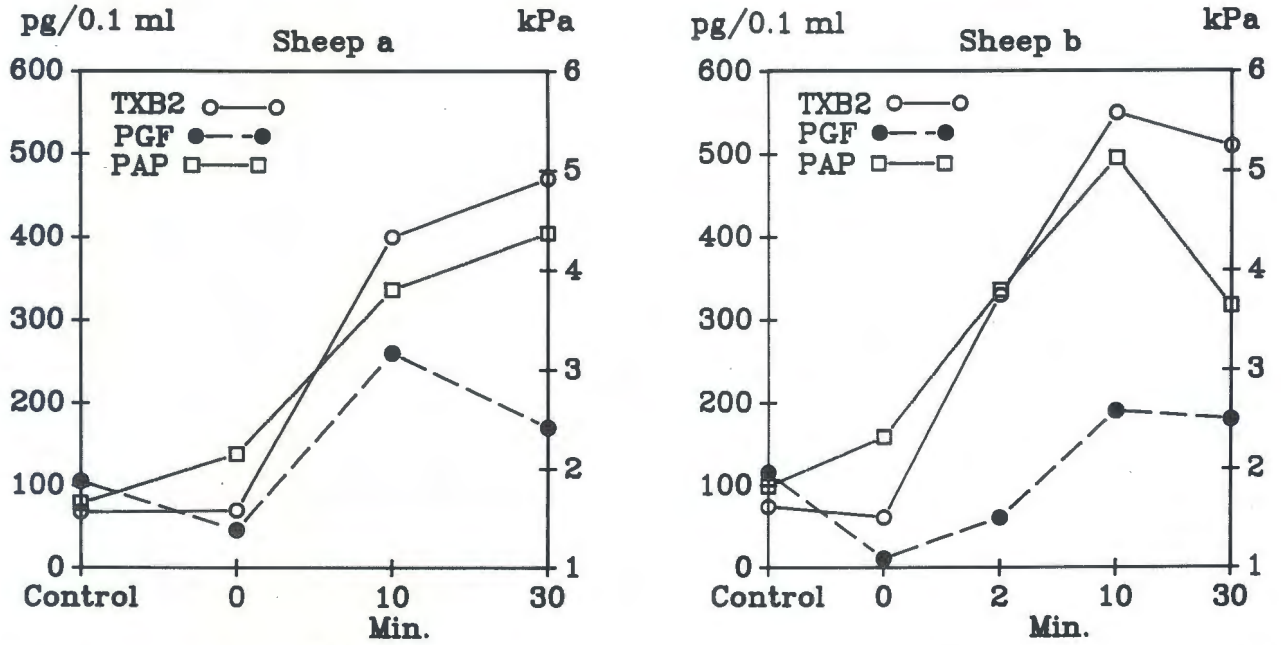

FIG. 3 Changes in thromboxane-B2 (TXB2) prostacycline (PGF) and pulmonary arterial pressure (PAP) in 2 sheep (a & b) as a result of a subcutaneous injection of 1 g of extracted fraction

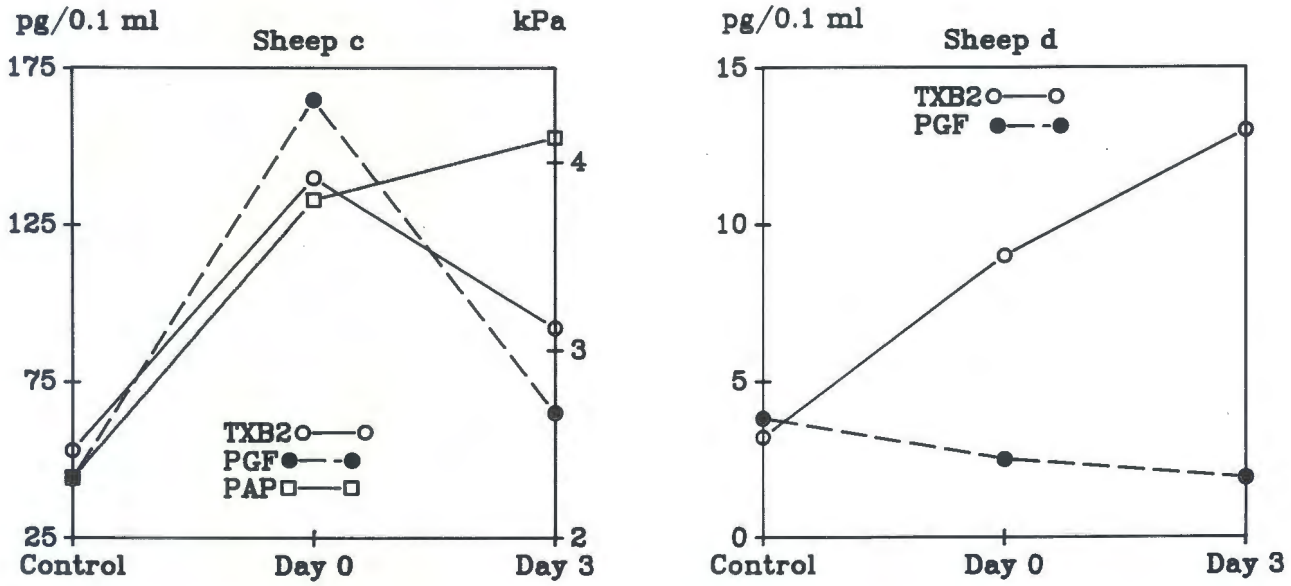

FIG. 4 Changes in prostaglandines and pulmonary arterial pressure for 2 sheep (c & d) dosed with plant material through rumen fistula. Day 0 = the start of decompensatory heart failure

determined by using test kits[2] from New England Nuclear.

RESULTS

An intravenous injection of the fraction extracted from *Pachystigma pygmaeum* resulted in significant changes in cardiac and pulmonary function. Fig. 1 shows some of the changes in the ECG and the pulmonary arterial pressure (PAP) which were usually induced by injection of the toxic fraction. Within 30 s, after the injection the PAP increased to values higher than 6,5 kPa, and ventricular extrasystoles could appear (Fig. 1b). With a small dosage of fraction the T wave becomes biphasic and then inverted. With a larger dosage, the level of the ST segment is shifted, and large T waves appear (Fig. 1c). These changes are typical of myocardial ischaemia. The animal used for this study collapsed when the ECG changed to that of Fig. 1 d. Of the 20 animals tested, all responded to intravenous injection of 2 mg–10 mg of the fraction with significant increases in PAP. In 18 of these animals the ECG also showed changes which varied from biphasic T waves to changes in the ST segment. All the animals were tested more than once to determine changes in their sensitivity for the injected fraction. One animal, which was used as a control, was injected 21 times at different time intervals.

The reaction of the animals to intravenous injection of the fraction did not depend only on the specific animal and the dosage used but also on the previous history of contact of the animal with the fraction. Animals which did not respond at all or

[2] Cat. no NEK-007 & NEK-008

showed only a slight response could be sensitized by regular intravenous or subcutaneous injection of a small dosage once a week. On the other hand, animals which responded could be desensitized if they received an injection every day or a progressively increasing dosage twice a week.

The physiological response of the cardiopulmonary system to the extracted fraction could be reduced or eliminated by intravenous adminstration of 300 mg of phenylbutazone[3]. Several animals which collapsed as a result of the fraction were saved by an injection of phenylbutazone. The ECG response obtained under nearly fatal conditions (Fig. 1d) was changed to a normal ECG within a few minutes after administration of phenylbutazone. It should however be emphasized that phenylbutazone dit not have any effect on chronic symptons or congestive heart failure which develop mostly after dosing sleep through rumen fistula (Joubert, 1985).

The influence of the extracted fraction on the cardiovascular system was studied quantitatively after subcutaneous administration in 4 animals. Heart catheterization was used to record the haemodynamic parameters. Approximately 5 h after the animals received the toxic fraction, a crisis developed with low stroke volumes and cardiac outputs, as well as high values for the CPFI, the pulmonary vascular resistance and the heart rate (Fig. 2). Beside the acute effects, some of the haemodynamic parameters remained abnormal for more than 30 h. In 3 of these animals the experiment was repeated after a few days and the same results were obtained. The duration and the intensity of the symptoms produced by the injected fraction are in favour of the hypothesis that the extract from *Pachystigma pygmaeum* contains the active ingredient which, when consumed during grazing, leads to irreversible damage of the myocardium after a latency period of approximately 4–8 weeks.

The ECG changes, which are an indication of severe myocardial ischaemia (Fig. 1c, d) and the disapperance of these symtoms with phenylbutazone, which is an inhibitor of prostaglandine synthesis, are indications that thromboxane and prostacycline may be involved in the actute reaction to the extracted fraction.

Thromboxane and prostacycline levels were determined in 4 sheep. For 2 of these animals the determinations were done shortly after subcutaneous injection of fraction, while for the other 2 plant material was dosed through rumen fistula and the prostaglandines were determined after haemodynamic symptoms of decompensatory heart failure had been observed. Fig. 3 shows changes in the serum levels of 6-keto-prostaglandine and thromboxane-B2, which are stable metabolic products of the unstable prostacycline (PGF2) and thromboxane-A2, as a result of a subcutaneous injection of 1 g of fraction. For both animals, large increases in these prostaglandines were observed during the first 30 min after the injection. During this time the pulmonary arterial pressures also rose to high values and correlated with the changes in thromboxane levels. For sheep dosed with plant material through rumen fistula, the prostaglandine levels were also changed. For 1 animal, both the thromboxane and prostacycline levels were elevated with the start of the decompensation phase of heart failure (Fig. 4a, Day 0), while for the other prostacycline did not change much while thromboxane kept rising during decompensatory failure (Fig. 4b).

Discussion

Several groups have succeeded in isolating a toxic fraction from rubiaceous plants (Veldsman, 1952; Anderson & De Kock, 1959; Potgieter, Jordaan, Cronje & Meij, 1975; Verschoor & Potgieter, 1984). While the aim of some studies with isolated fractions was to determine the molecular composition of the toxic compound, ours was to induce acute symptoms so that some of the different mechanisms which may be involved in gousiekte could be identified. Verschoor & Potgieter (1984) found that a dosage of 0,5–0,7 g/kg of a macromolecular fraction from plant juice was lethal when injected subcutaneously in a guinea-pig. Although toxicity was not determined by obtaining lethal dosage values, acute reactions were obtained in our experiments with 0,05 g/kg for subcutaneous injections in sheep.

The ECG changes observed with the extracted fraction correlated quantitatively with other results reported on gousiekte (Pretorius & Terblanche, 1967; Fourie *et al.*, 1989). The inverted T wave which was one of the criteria used by Fourie *et al.* (1989) for diagnosis of cardiac function was always observed in 1 of the ECG leads before the heart showed ischaemic ST segment shifts. The symptoms of myocardial ischaemia and myocardial arrhythmias, observed with the toxic fraction, may explain the sudden death often occurring with gousiekte without previous symptoms of congestive myocardial failure. A fatal episode of gousiekte such as this may occur if the level of the toxic substance rises as a result of an increased absorption or as a result of metabolic activation of the substance, if the animal becomes sensitized to the substance or as a result, myocardial arrhytmias which are typical of gousiekte.

The sensitization of the pulmonary arterial pressure response and the ECG changes when the toxic fraction is given at weekly intervals may be an indication that an immune mechanism is involved. Desensitization, on the other hand, may be due to the depletion of the vaso-active substances involved, when the toxic fraction is injected at daily intervals.

The extent of the changes in pulmonary vascular resistance as well as the ischaemic ECG changes induced can only be explained if it is assumed that a very potent vasoconstrictor substance is involved. The inhibitory effect of the prostaglandine inhibitor, phenylbutazone, prompted a study for the determination of the levels of thromboxane and prostacycline in serum of animals injected with the toxic fraction as well as for animals dosed with plant material. In both instances, changes were observed in these prostaglandines. Although the design of these experiments does not allow for a quantitative correlation between prostaglandine levels and physiological function, it indicates involvement of prostaglandines in the development of gousiekte, whether directly or indirectly. It must also be taken into consideration that, as thromboxane is a vasoconstrictor and prostacycline a vasodilator, the ratio of these 2 substances may be the important factor during the develpment of gousiekte.

The changes in haemodynamic parameters as a result of a subcutaneous injection of 2 g of toxic fraction show good correlation with changes observed with the development of gousiekte as a result of the dosing of plant material through rumen fistula

[3] "Butazolidin", Ciba-Geigy

(Van der Walt *et al.*, 1981). The decrease in stroke volume, while the pulmonary capillary wedge pressure remains relatively constant, is an indication of a decrease in myocardial contractility. This is confirmed by the increase in the CPFI, an indication of a decreased pump function of the heart (Van der Walt *et al.*, 1981; Van Rooyen *et al.*, 1989). If compensatory mechanisms are also impaired, this may eventually also lead to myocardial failure. The increased pulmonary arterial pressure and pulmonary vascular resistance, which are caused by pulmonary vasoconstriction, increase the afterload on the right ventricle, while it may also impair the filling of the left ventricle.

Until the mechanisms involved in gousiekte are identified, it would be difficult to make predictions from correlations in symptoms from a model where acute symptoms are evoked by injections of a toxic fraction and a model where chronic symptoms are obtained with administration of plant material through rumen fistula. Differences can be expected, especially as digestion in the alimentary tract may change the activity of the active substance. Verschoor & Potgieter (1984) found that fermentation of the plant extracts increased the toxicity and suggested that microbial digestion in the rumen may be required for obtaining effective toxicity.

The effects seen with the injection of a toxic fraction extracted form *Pachystigma pygmaeum* in sheep showed a good correlation with results obtained in sheep dosed with the plant material through rumen fistula. With the injected fraction, symptoms of acute myocardial ischaemia were recorded, and this may be responsible for the sudden death observed in some cases of gousiekte. The experimental results with thromboxane and prostacycline may indicate that these prostaglandines are involved in the development of gousiekte.

REFERENCES

ANDERSON, L. A. P. & DE KOCK, W. T., 1959. The occurrence of ursolic acid in several plants reported to be responsible for gousiekte in South Africa. *Journal of the South African Chemical Institute*, 12, 114–115.

FOURIE, N., SCHULTZ, R. A., PROZESKY, L., KELLERMAN, T. S. & LABUSCHAGNE, L., 1989. Clinical pathological changes in gousiekte, a plant-induced cardiotoxicosis of ruminants. *Onderstepoort Journal of Veterinary Research*, 56, 73–80.

JOUBERT, H. W., 1985. Simptome van 'n ekstrak uit *Pachystigma pygmaeum* op die skaap. M.Sc skripsie, P.U. vir C.H.O. 82 pp.

KELLERMAN, T. S., COETZER, J. A. W. & NAUDE, T. W., 1988. Plant poisonings and mycotoxicoses of livestock in Southern Africa. Cape Town: Oxford University Press. 243 pp.

POTGIETER, D. J. J., JORDAAN, E. CRONJE, I. & MEIJ, H. S., 1975. Some properties of a plant constituent responsible for heart failure in animals. *Proceedings of the 1st Congress of the South African Biochemical Society*, 74–75.

PRETORIUS, P. J. & TERBLANCHE, M., 1967. A preliminary study on the symptomatology and cardiodynamics of gousiekte in sheep and goats. *Journal of South African Medical Association*, 38, 29–53.

PROZESKY, L., FOURIE, N., NESER, J. A. & NEL, P. W., 1988. A field outbreak in Ile-de-France sheep of a cardiotoxicosis caused by the plant *Pachystigma pygmaeum* (Schltr) Robyns (Rubiaceae). *Onderstepoort Journal of Veterinary Research*, 55, 193–196.

THEILER, A., DU TOIT, P. J. & MITCHELL, D. T., 1923. Gousiekte in sheep. *Report on Veterinary Research, Union of South Africa*, 9 & 10, 9–105.

VAN DER WALT, J. J., VAN ROOYEN, J. M., CILLIERS, G. D., VAN RYSSEN, J. C. J. & VAN AARDE, M. N., 1981. Ratio of cardiopulmonary blood volume to stroke volume as an index of cardiac function in animals and in man. *Cardiovascular Research*, 15 (10) 580-587.

VAN ROOYEN, J. M. & VAN DER WALT, J. J., 1989. The regulation of the cardiopulmonary blood volume to stroke volume in sheep. *Cardiovascular Research*, 23 (5), 453–459.

VELDSMAN, D. P., 1952. Chemical investigation of *Pachystigma pygmaeum* Schlechter (Gousiektebossie). *Onderstepoort Journal of Veterinary Research*, 25, 113–120.

VERSCHOOR, J. A. & POTGIETER, D. J. J., 1984. Preparation and properties of a crude extract to guinea-pigs from *Pachystigma pygmaeum*, a plant causing heart failure in ruminants. *Onderstepoort Journal of Veterinary Research*, 51, 245–248.

WALKER, J., 1908/9. Gouw ziekte: A disease of sheep. *Reports of the Government Veterinary Bacteriologist of the Transvaal Department of Agriculture*, 74, 74–99.