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Assessment of Adrenal Function in Goat (*Capra hircus*)

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Abstract: To ascertain the physiological functions of the adrenal glands, single stage bilateral adrenalectomy was performed on six female Bengal goats (*Capra hircus*). Blood and urine samples were collected at regular interval in post-operative periods for estimation of different parameters. A radioimmunoassay detected an invariable drop in plasma total cortisol and also a downward course with erratic nature of the flurometrically assessed urinary total catecholamines represent the adrenal insufficiency among these animals. A steady post-operative weight loss was evident in all of the adrenalectomized goats. Other progressive symptoms like anorexia, asphyxia, muscular asthenia were profound like those which occur in other mammalian species. Increased melanogenesis was established by the urinalysis. Long duration surviving goats with adrenal insufficiency showed a marked discolouration and pigmentation of the skin. The average survival period for the adrenalectomized goats reported in this study was 168 h. The influence of certain factors obtaining in the experimental situation upon survival is discussed in some detail. The goat may survive in some instances and it might be an exception to the concept that adrenalectomy is fatal to the mammals.

Key words: Goat, adrenalectomy, cortisol, catecholamines, survival period

INTRODUCTION

Even though the Adrenal gland was brought forcefully to the attention of medical men about hundred and fifty years back by Addison (1955) and much speculation and patient inquiry have been devoted to them since that time, their functional significance has not yet been fully disclosed.

In order to explore this field, adrenalectomy, the surgical extirpation of the adrenal glands were proved to be the most authentic and accessible way. Although numerous experiments on adrenalectomy have been carried out on laboratory rodents (Castonguay, 2002; Tse and Debons, 1986), canine (Van Sluijs *et al*., 1995), equid (Slone *et al*., 1983), porcine (Park and Gagner, 1995) and ovine (Scobie and Hynd, 1993) model and also its established clinical application on human patients (Rodriguez *et al*., 1987); goat, the small ruminant of caprine family have hardly been considered at all in this field of study. The aim of this study is to develop a caprine model of adrenalectomy for a close look on adrenal insufficiency, which might shade a fundamental light to our knowledge of adrenal physiology especially in case of small ruminants. Observations of the animals in respect to body weight, appetite, activity and measurement of the pertinent hormonal profile of the body were made for a period before adrenalectomy and followed continually thereafter until death. It would be of great interest to know, for instance, whether and how there is a stepwise disruption of physiological mechanisms and for which of these can death be attributed. Results, which have been secured in this

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laboratory, appeared to justify at this time a consideration of the chief functions of the adrenal gland and accumulating evidences indicate the direction in which solution of the enigmatic physiological character of this gland may be found.

MATERIALS AND METHODS

Twelve healthy female Bengal goats (*Capra hircus*) of about same age (twelve months) with an average body weight of 7 kg were divided into two groups viz., control and experimental. The animals of the experimental group were bilaterally adrenalectomized (BADX) (Fig. 1) (Das *et al*., 2007). The control group of animals were also sham operated (SHAM-ADX) for better comparison of surgical effect between the groups. The animals received no post-operative supportive therapy except topical antibiotic and the dressing of surgical wound. All the animals were quartered in separate metabolic cages located in an air-conditioned animal house maintained at a constant temperature of 30°C. Food and water were supplied *ad libitum* throughout the experimental period. In this present study, entire experimental protocol along with feeding, watering and maintenance of the animals were as per the standard practice (Pandiyan *et al*., 2005).

The control and experimental animals were observed continuously day and night after surgery and the body temperature, respiration along with other symptoms were noticed at short intervals. Individual body weight was measured by floor balance (Avery, India) and the rectal temperature was also recorded by clinical thermometer (Hick's, India) at a regular interval.

Blood samples were collected from jugular vein for cortisol determination at 36, 48, 60, 72, 96, 120, 144 and 168 h after the adrenal glands were removed. Similar sample collection schedule was followed for the SHAM-ADX animals. Plasma was immediately separated by centrifugation (1200 g for 30 min) and stored at -20°C till analysis. Plasma total cortisol was quantitated by radioimmunoassay (RIA) using DSL-2100 ACTIVE™ Cortisol RIA Kit (Diagnostic Sys. Lab. Inc., Texus, USA). Urine samples were also collected at regular interval from the metabolic cages. Urinary total catecholamines were determined flurometrically (Turner, USA) by the method of Varley *et al*. (1988), modified by Nath and Nath (1990).

From the freshly collected urine of the BADX goats, Benzidine test (Oser, 1976) was performed for the detection of blood in the urine. For qualitative detection of the presence of melanin, samples of urine were subjected to expose in contact with air for few hours after urination. Ferric chloride test (Bauer, 1990) was also done for the confirmation of the presence of melanin pigment in urine.

The observations of the present study were subjected to statistical analysis by two way analysis of variance and mean significant difference was studied by Duncan Multiple Analysis Test (Das and Giri, 1986) using SPSS software (Version SPSS, 1999. SPSS 1 User's Guide; Release 10.0.1 ed. SPSS Inc. USA).

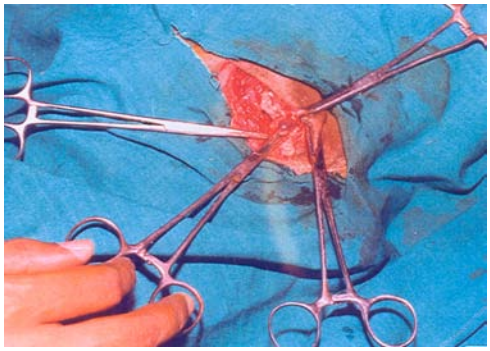

Fig. 1: Surgical adrenalectomy in Bengal goat

RESULTS

Recovery from the Trauma of Operation

The goats were adrenalectomized in the morning and within few hours no discernable effects of the operation were observed either on their general appearance or in their behaviour. They moved to their cages of its own with less agility than usual.

Symptomatology of the Adrenalectomized Goats

Less tangible but as striking were the changes in the general appearance and behaviour of the goats following adrenalectomy. As adrenal insufficiency progressed, the animals became less aggressive, frightened and more tractable when approached and handled. This was also observed in rough fashion, one of the commonly reported findings of adrenal insufficiency was anorexia. Food and water consumption was hard to measure in precision, however, was so gross as to be readily evident from 48th h onwards. Another usual finding of weight loss following adrenalectomy was consistent in those experimental goats. The results of the body weights of the pre and post-operative BADX as well as SHAM-ADX goats were presented in Fig. 2. Although non-significant, the over all result showed a tendency to maintain a lower body weight compared to the pre-operative stage. Pre-operative mean rectal temperature was recorded 39.22±0.13°C. A slight initial non-significant rise of body temperature (39.67±0.08°C) was noticed within next 48 h. Thereafter, the gradual decreasing trend of rectal temperature was succeeded by almost near normal value at the last phase of the experiment.

As adrenal insufficiency progressed, a decrease in the general muscle tone was felt and the animals were more inclined to sit quietly in the corner of their cages rather than their usual custom. Extreme muscular asthenia, prostration and very rapid and shallow respiration were the prominent symptoms from 72nd post-operative hours and onwards. This noticeable change in the respiratory pattern was almost maintained thereafter which sometime recorded a maximum respiratory rate of 26 per min. In the last stages of insufficiency convulsion followed by the seizures often tend to become continuous and sometimes ended in a convulsion of an obviously different, asphyxial character.

Duration of Survival Following Adrenalectomy

For the four among the six BADX goats reported in detail on this study, the average survival time without adrenal glands was 168 h. The other two goats have exceptionally survived over 30 days showing remarkable changes in different physiological parameters towards normal at last phase of the experiment.

In all cases of death careful post-mortem examinations were performed either immediately or within a few hours of death. Upon autopsy, no macroscopic pathology was visible in any of the major

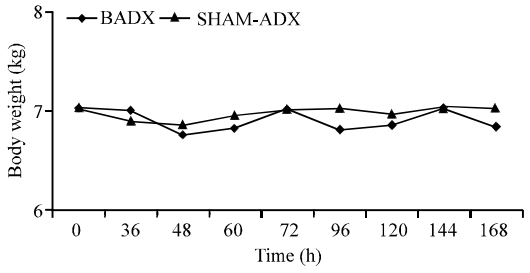

Fig. 2: Changes associated in body weight at different hours of BADX and SHAM-ADX goats

organs except some fluid accumulation in the thoracic cavity. Case of general peritonitis or any septic condition was not found; nor was there any post-operative hemorrhage. The heart and major arteries contained noticeable anti-mortem clot in three of the cases. No trace of adrenal gland could be recognized on either side, although a thorough examination was made. No trace of accessory glandular cortical tissue was detected near the original sites.

Changes in Hormonal Profile Following Adrenalectomy

Pre-operative (0 h) mean plasma cortisol value was 39.69±0.19 mmol L^{-1} (Fig. 3). Following adrenalectomy, the plasma concentration of cortisol was drastically decreased with a minimum of 8.38±0.44 mmol L^{-1} at 60th post-operative hour followed by a gradual increasing trend in later phase whereas the level was found to be significantly ($p<0.01$) elevated level in the major part of the study period with a maximum of 64.72±0.75 mmol L^{-1} at 36th h of sham operation. In case of the two long surviving BADX animals the concentration of plasma cortisol after one month was found to be an almost near normal value of 37.60±2.74 mmol L^{-1}, which is the most startling finding in this investigation.

The pattern of changes although an erratic one, statistically significant ($p<0.01$) difference exist among the concentrations of urinary catecholamines different hours (Fig. 4). Within the first 60 h of post adrenalectomy there was an increase in urinary catecholamines excretion up to 102.42±0.75 pmol L^{-1} followed by a sudden fall within next few hours. Thereafter a low level was maintained throughout the study with occasional increase in some cases. In case of SHAM-ADX animals urinary catecholamines were found to be always significantly ($p<0.01$) higher compared to the ADX goats.

Benzidine test of all the fresh urine samples showed the negative result. The urine, subjected to expose in contact of air, colour became slowly brown or dark coffee colour (Fig. 5), often from the surface to downwards, taking as long as 24 h or more with a marked distinction. Ferric chloride test

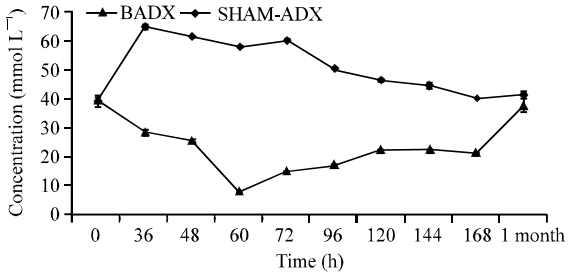

Fig. 3: Changes associated in plasma cortisol (nmol L^{-1}) at different hours of BADX and SHAM-ADX goats

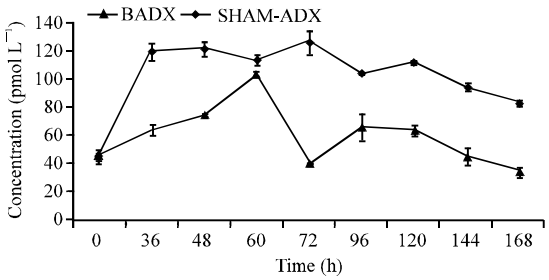

Fig. 4: Changes associated in urinary catecholamines (pmol L^{-1}) at different hours of BADX and SHAM-ADX goats

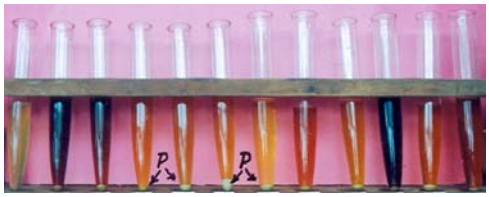

Fig. 5: Urine samples with precipitate (P) exposed to air for at least 1 h

Fig. 6: Skin pigmentation (P) of BADX goat surviving more than 1 month

of the fresh urine samples also gave positive results and after centrifuging (250 g for 5 min) the reacted samples showed a gray or black precipitation. The two exceptionally long surviving BADX goats showed a marked discolouration and pigmentation of skin of withers and crops region, revealed after close shaving of the hairs (Fig. 6).

DISCUSSION

The decline and eventual death of an animal following extirpation of the adrenal gland illustrates in an inimitable fashion the utter dependence of the organism on these glands for life. Observations of these animals give the impression of body wide disruption of function and this veritable plethora of disturbances impedes rather than assist an elucidation of adrenal gland physiology.

The distinctive post- operative weight loss in the present study can be interpreted by the evidence of anorexia, which was also previously reported by several workers working on different species (Tse and Debons, 1986; Castonguay, 2002). The loss of weight is not only that of loss of body tissue but loss of body fluid as well (Soffer *et al*., 1961). On several occasions, convulsion was observed which might be of either hypoglycemic origin (Kirchner *et al*., 2006) or related to the disturbances in various electrolytes, protein and carbohydrate metabolism (Soffer *et al*., 1961). The initial rise of body temperature at 48th post-operative hour might be due to enhanancement of body defense mechanism trying to cope up with the inflammatory process and surgical stress (Blood and Radostits, 1989).

Survival after bilateral adrenalectomy varies among individual animals and is influenced by diet, environment, genetic background, sex and age (Slone *et al*., 1983). The length of survival period following adrenalectomy is probably the resultant of many factors. Concentrating the fact of progressively altered haematological and serum electrolyte values in other BADX animal species (Wald *et al*., 1985; Scobie and Hynd, 1993) it seems more likely that the summated effect of several such coexistent abnormalities, such as severe inanition and haemodynamic upsets, for example, might proved to be fatal in this study also.

The two exceptional animals with both the adrenals completely removed have survived more than 30 days and would apparently have been indefinite, for the animal, so far as could be observed, was unaffected by the loss of glands. It is of course impossible to conclude, not withstanding the most careful post-mortem examination that the animal did not possess somewhere accessory adrenal glands, which vicariously took on the functions of the removed glands. Still we are inclined to regard such a solution of the survival as impossible, certainly there were no glandular structure visible to us at all resembling adrenals in either thorax or abdomen and nothing which gives a positive answer. Any possible accessory gland must, therefore, have been either microscopic in size or situated outside the regions where their existence was to be expected. Dukes (1955) stated, Accessory adrenals, consisting of either medullary or cortical tissue, or both, are not infrequent particularly in young animals. Histological evidence of the presence of accessory cortical tissue in long duration survived BADX-rats by Schwabedal and Partenheimer (1983) and similar evidence by Evans and Ortman (1995) in case of BADX-hampshire ewes supports our assumption of existence of accessory tissue.

Cortisol secretion varies with the time of the day in many species (Morimoto, 1982). The stresses of handling, vein puncture and disruption of sleep-wake cycle during sampling of the animals used in this study might have affected their cortisol secretion to certain extent. The increase in the cortisol value for SHAM-ADX goats in this study might be the reflection of surgical stress, whereas, following adrenalectomy plasma cortisol concentration decreased in maximum occasion might be due to severe adreno-cortical insufficiency in BADX-ADX goats, as because the major source of cortisol secretion is adrenal cortex. Similar type of low cortisol level was found in RIA method by Migeon and Lanes (1990) in primary adrenal insufficiency. At the last phase, the incremental level of plasma cortisol in case of the long surviving goats logically guides to the conclusion like many other workers (Hamada *et al*., 1982) that there might be any other source of cortisol in the body, which can be considered as a very impressive evidence for the development of accessory adrenocortical tissues in the BADX goats.

Catecholamines are considered to be a major determinant molecule for the physiological as well as psychological stress. It has been well established that these amines are secreted in excess during the stressed condition (Parrott *et al*., 1994). In the present study the initial sharp rise of urinary catecholamines might be indicative of post-surgical trauma associated with stress but from different source. Certain decreasing trend of urinary catecholamines level at several hours of investigation might be considered as an adrenal insufficiency as the major source of these amines is medulla part of the adrenal gland. But the erratic nature of level with some increasing trend at 120th h and the later part of the study might be the reflection of some extra adrenal epinephrine secretion which appeared to be a delayed response to removal of the adrenal medulla (Ricordi *et al*., 1988).

As the Benzidine test of all the freshly collected urine samples showed the negative result throughout the study, absence of hematuria and haemoglobinuria can be assumed. Dark or brown coffee colouration of urine might be due to the excess amount of colourless precursor melanogen, which was voided through fresh urine and oxidized to melanin in contact with air (Beeler and Herry, 1961). The urine samples having this nature might be considered as qualitative reflection of increased melanogen synthesis by the BADX goats. Another confirmatory evidence of this assumption is the positive result in the ferric chloride test with gray or black precipitation following centrifugation, is in accordance with the inference of Bauer (1990). Marked discolouration and pigmentation of the skin in adrenal insufficient human patient (Addission's disease) is very common and is due to the deposition of melanin in the skin and mucous membrane (Badri *et al*., 2007). In case of adrenalectomy similar type of pigmentation was also observed by Bronson and Clarke (1966) in deer mice and also by Rodriguez *et al*. (1987) in human patient. In the last phase of present study, the exceptionally long time survived adrenal insufficient goats represent a striking finding of increased melanogenesis with subsequent skin pigmentation.

In the light of earlier as well as the present evidences from this laboratory has salient features of adrenal insufficiency, especially in case of a small ruminant, goat. From the present study it can be concluded that the adrenal gland serves as one of the major endocrine organ in goat with such a wide range of physiological actions. A major handicap to maintain normal body weight in BADX goats is inadequate food intake. Muscular asthenia followed by prostration with asphyxia is the consequences of various metabolic disturbances inside the body. Plasma cortisol and urinary catecholamines profiles are also correlating with adrenal insufficiency. The presence of melanin in urine as well as skin pigmentation in later stage are guiding to the conclusion of increased melanogenesis. Removal of both adrenals in the goats caused death in only four out of six cases with an average survival period of 168 h. Death might take place due to cardio-respiratory failure along with severe disbalance of body homeostasis due to the absence of adrenal gland. Logicalistic assumption can be made regarding the development of accessory adrenal tissue elsewhere in the body, which has supported the life of the exceptionally long survived adrenalectomized goats.

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