Aust. J. Biol. Sci., 1974, **27**, 651–7

The Concentrations of Lipids in the Adrenal Cortical Tissue of Genetically Different Types of Cattle

J. C. O'Kelly

Division of Animal Genetics, CSIRO, Tropical Cattle Research Centre, Rockhampton, Qld 4700.

Abstract

The concentrations of lipids in the plasma and adrenal cortical tissue were compared in British (*Bos taurus*) and Zebu (Brahman cross and Africander cross) cattle. Plasma cholesterol and phospholipid concentrations were higher ($P < 0{\cdot}001$) in Zebu than in British animals. In grazing steers the adrenal cholesterol concentrations were higher ($P < 0{\cdot}01$) in Zebu than in British steers but phospholipid concentrations were lower ($P < 0{\cdot}01$) in Brahman cross than in Africander cross and British animals. The correlations between the concentrations of cholesterol in plasma and adrenal glands were significant. In Zebu steers on different dietary treatments the concentrations of cholesterol and phospholipid were higher ($P < 0{\cdot}01$) and triglyceride lower ($P < 0{\cdot}01$) in the adrenal tissue of steers growing normally than of steers losing weight. The adrenal to body weight ratios were lower ($P < 0{\cdot}01$) in Brahman cross than in Africander cross and British grazing animals.

Introduction

Adrenal cholesterol can be derived by local synthesis of cholesterol from acetate (Srere *et al.* 1948) but there is good evidence that plasma supplies the bulk of the metabolically active cholesterol in the rat (Morris and Chaikoff 1959; Dexter *et al.* 1970), dog (Krum *et al.* 1964) and man (Borkowski *et al.* 1967). Since the concentration of cholesterol is higher in the plasma of *Bos indicus* than in *B. taurus* cattle (O'Kelly 1968, 1972) the question was posed whether a similar difference exists in the concentration of cholesterol in the adrenal glands of these breeds. If so, this may be an important factor in the known superior ability of the *B. indicus* cattle to adapt to the stresses of a tropical environment. In this regard, large genetically determined differences in adrenal cholesterol ester concentrations have been reported in strains of mice (Doering *et al.* 1970) that had previously been observed to respond differently to stress in terms of plasma corticosterone concentrations (Levine and Treiman 1964).

This study investigated the adrenal weights and the concentrations of lipids in the plasma and adrenal glands of different breeds of cattle in a tropical environment.

Materials and Methods

The cattle used in this study grazed together at the National Cattle Breeding Station, 'Belmont', which is located 32 km north of Rockhampton. Mean annual rainfall in the area is 780 mm, of which over 60% falls in the four summer months, December–March. Mean maximum and minimum temperatures are 32 and 22°C respectively in January and 23 and 11°C respectively in July. Mean relative humidity is in the range 63–69%. The cattle used in the research program at Belmont include Brahman and Africander representing tropically adapted breeds and Herefords and Short-

horns as temperate (British) breeds. Helminths infesting the cattle include species of *Haemonchus*, *Cooperia*, *Oesophagostomum* and *Ostertagia*. Also, the cattle tick (*Boophilus microplus*) and buffalo fly (*Syphona exigua*) are endemic and seasonally active. The pastures are nutritionally adequate to support active growth of cattle in normal summer conditions but in the dry season the protein content of the pasture is very low. Supplementary feed is not given to the grazing animals in the dry season. One British and two Zebu breeds were used in the present experiments. The British animals were the progeny of matings of Shorthorn × Hereford and those referred to as Zebu were half-bred Brahman × British (Brahman cross) and half-bred Africander × British (Africander cross). The procedure used for the slaughter of these animals has been described by Seebeck (1973).

Table 1. Relation of adrenal weight to body weight and the concentrations of lipids in the plasma of steers on different dietary treatments (experiment 1)

Values given are means ± standard errors

Parameter	Animals gaining weight: Brahman cross	Animals losing weight: Brahman cross	Africander cross
Liveweight (kg)	355±19	342±12	332±8
Left adrenal weight (g)	5·72±0·61	5·22±0·40	5·68±0·27
Right adrenal weight (g)	6·52±0·62	5·66±0·32	5·04±0·31
Adrenal : body weight (g/100 kg)	3·45±0·30	3·18±0·13	3·23±0·15
Plasma lipids			
Total cholesterol (mg/100 ml)	113·8±7·3	76·4±10·2	79·0±3·5
Phospholipid (mg/100 ml)	118·7±6·1	80·1±3·5	78·4±4·0

Experiment 1. Effect of Body Weight Loss on the Lipid Composition of Plasma and Adrenal Cortical Tissue of Steers

A total of 14 Zebu steers was used. One group (A) of four Brahman cross steers was allowed to graze good quality pasture to achieve normal growth until slaughter at weights ranging from 330 to 368 kg. Another group (B), comprising four Brahman cross and six Africander cross steers, were grazed in the same paddock as group A until they reached 390 kg body weight. They were then transferred to stalls and fed low-quality hay and straw such that they might lose approximately 0·5 kg body weight per day until slaughter at weights ranging from 315 to 356 kg. The steers were born in November–December 1965 and were slaughtered over the period November 1967 to March 1968.

Experiment 2. Lipid Composition of the Plasma and Adrenal Cortical Tissue of Grazing Steers

Two trials were conducted and in each 11 Brahman cross, 11 Africander cross and 11 British steers were used. The same experimental design was used in both trials and was such that animals of the three breed groups selected for slaughter were within the same liveweight range. The steers in the first trial were born in November–December 1966 and were slaughtered over the period November 1968 to August 1969; those in the second trial were born in November–December 1967 and were slaughtered during the period March 1970 to January 1971.

Two additional animals were used to obtain the adrenal glands in the minimum time after death. These animals were not dissected for carcass composition studies but were cut through to the adrenal gland region immediately after slaughter.

Analytical Procedures

Blood samples were collected from each animal at slaughter. Then the adrenal glands from each side were removed, cleaned from adhering fat and weighed. The cortical and medullary tissues, which were quite distinct, were easily separated by dissecting with a scalpel. A portion of cortical tissue of each gland was cut and weighed, then extracted for lipids. The techniques used for the analyses of cholesterol, phospholipid, triglyceride and total lipid have been described previously (O'Kelly 1974).

The sum of the weights of the left and right adrenal glands was used in calculating the adrenal to body weight ratios. The results were analysed by analysis of variance.

Results

Experiment 1

(1) *Adrenal weights.* Although the mean weights of the adrenal glands and the adrenal to body weight ratio were higher in the steers gaining weight than in the steers losing weight (Table 1) the differences were not statistically significant.

(2) *Plasma lipids.* The plasma concentrations of cholesterol and phospholipid were significantly higher ($P < 0{\cdot}01$) in the Brahman cross steers gaining weight than in the two groups of steers losing weight (Table 1).

Table 2. Lipid concentrations in the adrenal cortical tissue of steers on different dietary treatments (experiment 1)

Values given are means ± standard errors, and are expressed as mg/g wet tissue

Parameter	Adrenal gland	Animals gaining weight: Brahman cross	Animals losing weight: Brahman cross	Africander cross
Total cholesterol	Left	3·60±0·13	1·86±0·22	1·96±0·24
	Right	3·48±0·11	1·73±0·03	1·94±0·20
Free cholesterol	Left	3·42±0·18	1·79±0·20	1·76±0·22
	Right	3·31±0·12	1·62±0·08	1·72±0·21
Free : total cholesterol (%)	Left	95·1±2·6	96·1±1·2	89·8±2·9
	Right	95·2±2·4	93·8±2·9	88·9±3·1
Phospholipid	Left	20·06±0·80	10·00±0·98	11·12±1·00
	Right	19·98±0·28	9·82±0·48	10·96±1·12
Triglyceride	Left	0·84±0·12	6·96±0·92	6·90±1·05
	Right	0·82±0·27	6·74±1·03	6·67±1·10
Total lipid	Left	24·50±1·04	18·82±0·86	19·98±0·41
	Right	24·28±0·50	18·29±0·90	19·57±0·62

(3) *Adrenal cortical tissue lipids.* The concentrations of total and free cholesterol, phospholipid and total lipid in the adrenal cortical tissue (Table 2) were significantly higher ($P < 0{\cdot}01$) in the Brahman cross steers gaining weight than in both the Brahman and Africander cross animals losing weight. The reverse was found for the concentrations of triglycerides, which were higher ($P < 0{\cdot}01$) in Africander cross and Brahman cross animals losing weight than in the Brahman cross steers gaining weight. These effects of treatment were similarly expressed in both left and right adrenal glands. Treatment was without effect on the proportion of cholesterol present in the free state. Within treatment groups there were no significant differences between left and right adrenal glands in the concentrations of lipids or in the ratio of free to total cholesterol.

Experiment 2

(1) *Adrenal weights.* Within each breed group the body weights and adrenal weights were not significantly different between trials 1 and 2 (Table 3) and the combined values for both trials were used for comparisons between breeds.

The weights of the left and right adrenal glands and the adrenal to body weight ratio of the Brahman cross steers were significantly lower ($P < 0{\cdot}01$) than those of the Africander cross and British steers. In the Brahman cross animals the weight of the right adrenal gland was higher ($P < 0{\cdot}05$) than the left, but the reverse was found in the other two breed groups. Correlations between adrenal weight and body weight were significant ($P < 0{\cdot}01$) in Brahman cross ($r = 0{\cdot}845$), Africander cross ($r = 0{\cdot}735$) and British ($r = 0{\cdot}846$) breeds.

Table 3. Adrenal weights and relation of adrenal weight to body weight in grazing steers (experiment 2)
Values given are means ± standard errors

Parameter	Trial	Brahman cross	Africander cross	Shorthorn × Hereford
Liveweight (kg)	1	396±20	397±19	387±18
	2	400±11	402±12	398±17
Left adrenal weight (g)	1	5·84±0·21	8·66±0·42	8·18±0·40
	2	5·82±0·14	8·78±0·33	8·39±0·21
Right adrenal weight (g)	1	6·44±0·25	7·90±0·10	7·41±0·23
	2	6·50±0·08	7·82±0·22	7·53±0·24
Adrenal : body weight (g/100 kg)	1	3·10±0·06	4·17±0·19	4·03±0·12
	2	3·08±0·05	4·13±0·16	4·00±0·10

Table 4. Concentrations of lipids in plasma and adrenal cortical tissue of grazing steers (experiment 2)
Values given are means ± standard errors

Parameter	Trial	Brahman cross	Africander cross	Shorthorn × Hereford
Plasma lipids (mg/100 ml)				
Total cholesterol	1	136·8±4·2	129·4±6·1	89·1±2·1
	2	126·6±6·1	128·7±3·8	87·9±4·2
Phospholipid	1	149·1±3·5	139·5±4·6	96·0±1·9
	2	141·5±7·0	128·6±2·3	90·3±4·2
Lipids in adrenal cortical tissue (mg/g wet tissue)				
Total cholesterol	1	4·10±0·10	4·08±0·10	3·51±0·07
	2	4·16±0·12	3·98±0·08	3·45±0·09
Free cholesterol	1	3·88±0·11	3·93±0·11	3·27±0·09
	2	3·95±0·14	3·76±0·09	3·15±0·10
Free : total cholesterol (%)	1	94·6±2·0	96·4±1·1	93·1±3·0
	2	95·0±1·8	94·6±2·8	91·4±2·9
Phospholipid	1	14·24±0·26	16·41±0·27	16·80±0·42
	2	14·19±0·41	16·56±0·45	16·72±0·61

(2) *Plasma lipids.* The plasma concentrations of cholesterol and phospholipid were significantly higher ($P < 0{\cdot}01$) in Zebu than in British steers in both trials (Table 4).

(3) *Adrenal cortical tissue lipids.* Within each breed there were no significant differences in the concentrations of lipids between the left and right adrenal glands, and the values shown in Table 4 are the mean values for both glands. Also, there

were no within-breed differences in the concentrations of lipids between trials 1 and 2 and the combined values for both trials were used for comparisons between breeds.

The concentrations of total and free cholesterol were higher ($P < 0{\cdot}01$) in Zebu than in British steers but there were no breed differences in the ratio of free to total cholesterol. The concentration of phospholipid was lower ($P < 0{\cdot}01$) in Brahman cross than in Africander cross and British animals.

The within-breed correlation between the concentrations of total cholesterol in plasma and adrenal cortical tissue was significant ($r = 0{\cdot}427$, $P < 0{\cdot}01$).

In the two additional animals studied the adrenal glands were removed within 5 min after slaughter. All the cholesterol in the four adrenal glands removed from these animals was in the free form.

Discussion

This study has established that the ratio of adrenal to body weight is significantly lower in Brahman cross than in Africander cross and British steers grazing in a tropical environment. The differences in adrenal gland size may reflect differences in reaction to the environment. Rats bred for several generations in conditions of high temperature and humidity have been reported to show a relative increase in adrenal gland size and continuous hyperactivity of the adrenal gland also results in its enlargement (Tepperman *et al.* 1943). The adrenal glands of animals exposed to tropical conditions show morphological as well as histological and histochemical changes (Schmidt and Schmidt 1938; Flexner and Grollman 1939; Bernstein 1941). Since British animals are less heat-tolerant than Brahman cross ones, a plausible explanation for the breed difference would be an increased gland size in the British animals resulting from continual exposure to conditions of heat stress. This would not, however, explain the higher adrenal weight of the Africander cross steers, as they are nearly as heat-tolerant as the Brahman cross ones. Other stressful factors may be involved. Brahman cross cattle have a high resistance to internal parasites, but Africander cross are nearly as susceptible as British breeds (Seifert 1971). It may be that parasitic burdens are factors influencing adrenal gland size.

However, adrenal weights may not always be valid indices of function due to factors which include (1) the presence of immature zones (X-zones), since there is little evidence that such zones contribute to cortical function, and (2) the possibility of weight loss with excessive stimulation and a misleading increase in adrenal weight due to accumulation of 'infiltrated' lipids. An eightfold increase in the concentrations of triglyceride in the adrenal glands of cattle losing weight through inadequate nutrition was demonstrated in this study.

Histochemical studies on the adrenal cortex of *Bos taurus* showed that phospholipids comprised the major part of the relatively small quantity of lipids and that lipoproteins were, for the greater part, responsible for the diffuse sudanophilia all over the cortex (Gospodinow and Kietz 1967). The results reported here confirm the low total cholesterol and phospholipid concentrations in bovine adrenal glands (Parhow and Cahane 1931; Brown *et al.* 1937; Cargill and Cook 1964), but demonstrate for the first time in cattle the dependence of the adrenal cortical lipid content on the nutritional status of the animal.

It was shown that in cattle on an inadequate diet the fall in plasma total cholesterol and phospholipid was accompanied by a decrease in the concentrations of these two

lipid constituents in the adrenal cortex. This may imply an inadequate adrenal reserve and suggests that in undernutrition the adrenal cortex may become less responsive to stress stimuli and result in depression of the secretion rate of the gland. This reasoning may also be extended to explain the breed differences in the concentrations of plasma and adrenal cortical cholesterol and reactions to stressful environments; the lower amounts of cholesterol in the British compared to the Zebu steers may reflect a lower utilization of cholesterol from plasma for steroidogenic activity of the adrenal gland. However, no explanation is offered for the lower concentration of phospholipid in the Brahman cross steers (Table 4) as there is no evidence that phospholipid is involved in the process of steroidogenesis.

No cholesterol esters were found in the adrenal cortex of cattle by Sorg and Jaffé (1924), and calculations from the data presented by Brown *et al.* (1937) revealed that the cholesterol present as the ester fraction was about 10% of the total cholesterol in beef cortical tissue. The present results confirm the low values of cholesterol esters in ruminants as compared with the high values (80–90%) found in other mammals including rat, rabbit and man (Goodman 1965). Cholesterol esterifying activity was found to be deficient in homogenates of bovine adrenal tissue (Longcope and Williams 1963). In contrast, Glick and Ochs (1955) reported that 50% of the total cholesterol of bovine adrenal glands is esterified and suggested that the stress during slaughter may be responsible for the low value of sterol generally reported and also that postmortem hydrolysis decreases the esterified sterol. However, removal of the adrenal glands from two animals within 5 min of slaughter in the present study showed that all the cholesterol was in the free form.

The extent to which the cholesterol esters contribute to the dynamics of hormone production has not been well investigated. It appears that in man and laboratory animals hydrolysis of esters must precede the conversion of sterol into steroids and that cholesterol esters can act as a reservoir of steroid precursors (Dailey *et al.* 1963; Grant *et al.* 1968). The lack of cholesterol esters in bovine adrenal cortical tissue does not support the hypothesis of a cholesterol ester pool from which cholesterol for steroidogenesis may be rapidly obtained.

Acknowledgments

The author is grateful to Dr R. M. Seebeck for use of his experimental facilities; to Mr H. G. Turner for helpful criticism of the manuscript; and to Mrs H. Komdeur and Miss K. Cagney for technical assistance. The work was supported in part by the Australian Meat Research Committee.

References

Bernstein, J. G. (1941). The effect of thermal environment on the morphology of the thyroid and adrenal cortical glands in the albino rat. *Endocrinology* **28**, 985–98.

Borkowski, A. J., Levin, S., Delcroix, C., Mahler, A., and Verhas, V. (1967). Blood cholesterol and hydrocortisone production in man: quantitative aspects of the utilization of circulating cholesterol by the adrenals at rest and under adrenocorticotrophin stimulation. *J. Clin. Invest.* **46**, 797–811.

Brown, J. B., Knouff, R. A., Conlin, M. M., and Schneider, B. M. (1937). Comparative lipid analysis of cortex and medulla of beef adrenals. *Proc. Soc. Exp. Biol. Med.* **37**, 203–5.

Cargill, D. L., and Cook, R. P. (1964). The free and esterified sterol present in bovine adrenal cortex and medulla. *Biochem. J.* **93**, 504–12.

Dailey, R. E., Swell, L., and Treadwell, C. R. (1963). Hydrolysis and utilization of cholesterol esters for steroid synthesis by canine adrenal homogenates. *Arch. Biochem. Biophys.* **100**, 360–3.

Dexter, R. N., Fishman, L. M., and Ney, R. L. (1970). Stimulation of adrenal cholesterol uptake from plasma by adrenocorticotrophin. *Endocrinology* **87**, 836–46.

Doering, C. H., Kessler, S., and Clayton, R. B. (1970). Genetic variation of cholesterol ester content in mouse adrenals. *Science* (*Wash. D.C.*) **170**, 1220–2.

Flexner, L. B., and Grollman, A. (1939). The reduction of osmic acid as an indicator of adrenal cortical activity in the rat. *Anat. Rec.* **75**, 207–21.

Glick, D., and Ochs, M. J. (1955). Studies in histochemistry: quantitative histological distribution of cholesterol in adrenal glands of the cow, rat and monkey and effects of stress conditions, ACTH, cortisone and desoxycorticosterone. *Endocrinology* **56**, 285–98.

Goodman, D. S. (1965). Cholesterol ester metabolism. *Physiol. Rev.* **45**, 747–839.

Gospodinow, C., and Kietz, D. (1967). Histochemical studies on lipids in adrenal cortex of *Bos taurus*. *Acta Histochem.* **27**, 107–16.

Grant, J. K., Griffiths, K., and Lowe, M. (1968). Biochemical investigations of the actions of corticotrophins on the adrenal cortex. *Mem. Soc. Endocrinol.* **17**, 113–24.

Krum, A. A., Morris, M. D., and Bennett, L. L. (1964). Role of cholesterol in the *in vivo* biosynthesis of adrenal steroids by the dog. *Endocrinology* **74**, 543–7.

Levine, S., and Treiman, D. M. (1964). Differential plasma corticosterone response to stress in four inbred strains of mice. *Endocrinology* **75**, 142–4.

Longcope, C., and Williams, R. H. (1963). Cholesterol esterification by adrenal homogenates. *Proc. Soc. Exp. Biol. Med.* **113**, 754–6.

Morris, M. D., and Chaikoff, I. L. (1959). The origin of cholesterol in liver, small intestine, adrenal gland and testis in the rat: dietary versus endogenous contributions. *J. Biol. Chem.* **234**, 1095–7.

O'Kelly, J. C. (1968). Comparative studies of lipid metabolism in Zebu and British cattle in a tropical environment. I. Plasma lipid levels of grazing cattle. *Aust. J. Biol. Sci.* **21**, 1013–24.

O'Kelly, J. C. (1972). Seasonal variations in the plasma lipids of genetically different types of cattle: grazing steers. *Comp. Biochem. Physiol.* **43B**, 283–94.

O'Kelly, J. C. (1974). The concentrations of lipids in the plasma, liver and bile of genetically different types of cattle. *Comp. Biochem. Physiol.* (In press.)

Parhow, C. I., and Cahane, M. (1931). Sur la teneur en cholesterol et en eau de l'ecorce surrenale chez quelques espèces de mammiferes. *C. R. Seances Soc. Biol.* **107**, 836–7.

Schmidt, I. G., and Schmidt, L. H. (1938). Variations in the structure of adrenals and thyroids produced by thyroxine and high environmental temperatures. *Endocrinology* **23**, 559–65.

Seebeck, R. M. (1973). The effect of body-weight loss on the composition of Brahman cross and Africander cross steers. *J. Agric. Sci.* **80**, 201–10.

Seifert, G. W. (1971). Ecto- and endoparasitic effects on the growth rates of Zebu crossbred and British cattle in the field. *Aust. J. Agric. Res.* **22**, 839–50.

Sorg, K., and Jaffé, R. (1924). Lipoiduntersuchungen an den nebennieren des rindes. *Zentralbl. Allg. Pathol. Pathol. Anat.* **35**, 353–9.

Srere, P. A., Chaikoff, I. L., and Dauben, W. G. (1948). The *in vitro* synthesis of cholesterol from acetate by surviving adrenal cortical tissue. *J. Biol. Chem.* **176**, 829–33.

Tepperman, J., Engel, F. L., and Long, C. N. H. (1943). A review of adrenal cortical hypertrophy. *Endocrinology* **32**, 373–402.

Manuscript received 10 May 1974