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A TEST OF ADRENAL CORTICAL FUNCTION IN DAIRY COWS BY ACTH ADMINISTRATION

By

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The possibilities of determining the adrenal cortical activity in ruminants in a relatively simple way are at present very limited. The methods introduced in human medicine for determination of the urinary excretion of corticoids or corticoid metabolites are almost without exception unusable on cattle (*Holtz* 1956; *Holcombe* 1957). *Holcombe* reported a practicable method of estimating the urinary "reducing corticoids", but it is too complicated for routine laboratory use. Still more does this apply to the methods used for determining the content of 17-hydroxy-corticosteroids in the blood of ruminants (*Robertson & Mixner* 1956; *Brush* 1958; *Lindner* 1959). The possibilities of estimating the adrenal cortical function in cattle by measuring the excretion of Na and K in urine would seem to be limited by the normally great variation in healthy cows (*Pehrson* in press).

Thorn et al. (1948) introduced a test of the adrenal cortical function in man, based on certain physiological consequences of intramuscular injection of the pituitary hormone ACTH. They considered that a more than 50 per cent decrease of the circulating eosinophils in blood and a more than 50 per cent increase of the uric acid — creatinine ratio eliminates practically all likelihood of adrenal cortical insufficiency. A reduction of the eosinophils after an injection of ACTH has later been found to occur in several animal species, and this has been used in veterinary medicine on rather dubious grounds as a test of adrenal cortical activity. This diagnostical test has often been called the *Thorn* test, which is actually a misnomer for, as far as we know, no one

within veterinary medicine has determined the uric acid — creatinine ratio in this connection, as was done in the test originally introduced by *Thorn et al.*

The normal changes of the bovine white blood picture caused by an ACTH injection have been studied by several authors. *Andersson* (1954) found, that after intramuscular ACTH injections of varying strength the most pronounced changes occurred after 6—8 hours. The number of eosinophils diminished, but at the same time the neutrophils and the total white count increased. The number of lymphocytes was unchanged. The blood sugar content increased if the ACTH injection was more than 100 i.u. *Hopwood & Tibolla* (1958) also reported a decrease in the number of eosinophils circulating in the blood, being most pronounced, however, 10 hours after the intramuscular injection. *Köhler* (1960) found the greatest effect usually after 8 hours; at that time there had occurred an increase in the percentage of neutrophils and a decrease in the percentage of eosinophils and lymphocytes. In the investigations of *Meyer* (1962) the most pronounced changes in white blood picture occurred after 8—10 hours and consisted in an increase of total white count and neutrophils and in a decrease of eosinophils and lymphocytes.

Weirich (1960) found that the maximal effect of an intravenous ACTH injection of 40 i.u. occurred after 4—6 hours. Of 40 clinically healthy cows only 65 per cent showed a more than 50 per cent decrease of eosinophils at that time, i. e. only 65 per cent of the cows responded positively to the Thorn test on the principles of assessment then accepted in veterinary medicine.

Ziel (1963) injected 40 i.u. of ACTH intravenously and, in about 250 cattle, checked the effect on the eosinophil count 4—6 hours after the injection. The decrease in the eosinophil count that had then occurred varied very much in different cattle and on different sampling occasions. As criterion of a positive Thorn test *Ziel* used that recommended by *Thorn et al.* (1948) for man, i. e. a positive response from the adrenal cortex to an ACTH injection causes at least a 50 per cent decrease in the number of eosinophils circulating in the blood. *Ziel* considered that the test in its original form could not be used in cattle, as he found too many negative responses in clinically healthy animals.

In the literature studied by us there are reports that the blood content of eosinophils in untreated cattle varies with the time of day (*Karg* 1955; *Weirich* 1960; *Ziel* 1963). All these authors

reported an increase from morning to afternoon or evening. *Moberg* (1955), on the contrary, found no variation with the time of day.

Several authors have reported variations in the intensity of reaction to an ACTH injection in different cows. Individual variations (*Andersson* 1954; *Weirich* 1960; *Ziel* 1963) and a decrease in reaction with increasing age (*Weirich* 1960; *Ziel* 1963) are said to be of importance in this connection. Concerning the importance of milk productivity there are two contradictory reports (*Weirich* 1960; *Ziel* 1963).

In the literature at our disposal we have not found any reports on side reactions to an ACTH injection in cattle, though *Sjögren* (1962) reported that serious allergic reactions sometimes occur in man.

MATERIAL AND METHODS

The animals used for the investigation were high-lactating, clinically healthy cows of the Swedish Red and White Breed (SRB), all from the same herd. The age varied from 3 to 11 years, and all tests were made between the 7th and 100th day after parturition.

The ACTH test was performed as follows. Two initial blood samples were taken at an interval of one hour (at 8 and 9 o'clock in the morning), and thereafter 200 i.u. of ACTH*) were given intravenously. Three more blood samples were taken 6, 8 and 10 hours after injection. These times were chosen because preliminary investigations had shown that the maximal effect was not reached until after 6 hours and because sampling more than 10 hours after injection as a rule involves practical difficulties. In the control tests the same scheme was followed except that no ACTH injection was performed. With two exceptions each cow was used for one ACTH test as well as one control test, at intervals of at least 4 days.

The total white blood count was determined in a Bürker counting chamber (0.4 mm³ of blood, diluted 1:20), while differential counts of at least 400 cells from each sample were performed on two smears stained with May-Grünwald's solution. The numbers of lymphocytes, neutrophils and eosinophils were determined, as well as the percentage of each. Monocytes were

*) Acton prolongatum, Ferring, Malmö.

Table 1. The total white count per mm³ before (initial sample) and 6, 8 and 10 hours after intravenous administration of 200 i.u. of ACTH. No ACTH injection in control test.

	ACTH test (n = 15)				Control test (n = 13)			
	Initial sample	+ 6 h	+ 8 h	+ 10 h	Initial sample	+ 6 h	+ 8 h	+ 10 h
\bar{x}	7193	9440	8700	8807	7015	7500	7992	7231
s	2541	3176	2835	2842	2678	2341	3518	2380
t	—	2.14*	1.53	1.64	—	0.49	0.80	0.22

Table 2. The number of *lymphocytes* per mm³ before (initial sample) and 6, 8 and 10 hours after intravenous administration of 200 i.u. of ACTH. No ACTH injection in control test.

	ACTH test (n = 15)				Control test (n = 13)			
	Initial sample	+ 6 h	+ 8 h	+ 10 h	Initial sample	+ 6 h	+ 8 h	+ 10 h
\bar{x}	5576	5584	5106	5083	5397	5598	5907	5210
s	2413	2698	2488	2569	2905	2386	2660	1984
t	—	0.01	0.53	0.54	—	0.19	0.47	0.19

Table 3. The number of *neutrophils* per mm³ before (initial sample) and 6, 8 and 10 hours after intravenous administration of 200 i.u. of ACTH. No ACTH injection in control test.

	ACTH test (n = 15)				Control test (n = 13)			
	Initial sample	+ 6 h	+ 8 h	+ 10 h	Initial sample	+ 6 h	+ 8 h	+ 10 h
\bar{x}	1154	3633	3441	3585	1169	1472	1565	1580
s	256	1123	1192	953	431	568	882	733
t	—	8.34***	7.27***	9.54***	—	1.53	1.46	1.74

Table 4. The number of *eosinophils* per mm³ before (initial sample) and 6, 8 and 10 hours after intravenous administration of 200 i.u. of ACTH. No ACTH injection in control test.

	ACTH test (n = 15)				Control test (n = 13)			
	Initial sample	+ 6 h	+ 8 h	+ 10 h	Initial sample	+ 6 h	+ 8 h	+ 10 h
\bar{x}	457	223	164	156	439	441	530	462
s	251	226	115	146	106	188	199	110
t	—	2.69*	4.12***	4.03***	—	0.04	1.45	0.55

counted as lymphocytes, while the few basophils were disregarded. All counts were made by the same person.

Blood sugar determinations were made according to Hagedorn-Jensen.

The statistical analysis was performed according to Student's t-test*). As no statistically significant difference was found in any respect between the two initial samples (at 8 and 9 o'clock), the mean of the two values was used in the calculations. Only the t-values found on comparison between initial samples and the respective post-injection samples are given. On grounds of space the t-values found on internal comparison between post-injection samples (+6, +8, +10 hours) are not given in the tables, as no statistically significant differences were found.

RESULTS

1. *The total white count* (Table 1) showed a clearly rising tendency after the ACTH injection. In the control tests no significant changes were found.
2. *The lymphocytes* (Table 2) showed no significant change, either on ACTH administration or in the control experiments. The percentage of lymphocytes in the differential count, however, showed a significant (***) decrease after ACTH injection (Table 5). This is explained by the large increase of the number of neutrophils (see below).
3. *The neutrophils* increased significantly (***) after ACTH administration, both numerically (Table 3) as well as percentually (Table 5). In the control tests there was an increasing tendency from morning to afternoon and evening (Table 3).
4. *The eosinophils* decreased significantly (*-***) after ACTH administration, both numerically (Table 4) and percentually (Table 5). The number of eosinophils showed no significant changes at different times of the day in the control tests.
5. As regards *the differential white count* (Table 5) a change occurred on ACTH administration, the percentage of lymphocytes and eosinophils decreasing and the percentage of neutrophils

*) n = number of samples; s = standard deviation; \bar{x} = mean.

* 0.05 > P > 0.01; ** 0.01 > P > 0.001; *** P < 0.001.

Table 5. *The percentage of lymphocytes, neutrophils and eosinophils before (initial sample) and 6, 8 and 10 hours after intravenous administration of 200 i.u. of ACTH. No ACTH injection in control test.*

ACTH test (n = 15)												
Initial sample			+ 6 h			+ 8 h			+ 10 h			
Lympho- cytes	Neutro- phils	Eosino- phils	Lympho- cytes	Neutro- phils	Eosino- phils	Lympho- cytes	Neutro- phils	Eosino- phils	Lympho- cytes	Neutro- phils	Eosino- phils	
\bar{x}	76.1	17.4	6.5	57.7	40.0	2.3	57.0	41.1	1.9	55.5	42.9	1.6
s	5.1	5.4	3.6	11.6	12.8	2.4	13.7	13.8	1.6	12.1	12.4	1.6
t	—	—	—	5.67***	6.31***	3.72***	5.07***	6.19***	4.45***	6.07***	7.32***	4.79***

Control test (n = 13)												
Initial sample			+ 6 h			+ 8 h			+ 10 h			
Lympho- cytes	Neutro- phils	Eosino- phils	Lympho- cytes	Neutro- phils	Eosino- phils	Lympho- cytes	Neutro- phils	Eosino- phils	Lympho- cytes	Neutro- phils	Eosino- phils	
\bar{x}	74.5	18.6	6.9	73.2	20.7	6.1	73.3	19.9	6.8	71.2	22.4	6.4
s	9.9	8.2	2.5	9.5	8.4	2.4	7.3	6.0	1.7	8.4	7.7	1.6
t	—	—	—	0.32	0.64	0.88	0.34	0.46	0.18	0.90	1.21	0.56

Table 6. *The blood sugar content in mg per 100 ml before (initial sample) and 6, 8 and 10 hours after intravenous administration of 200 i.u. of ACTH. No ACTH injection in control test.*

	ACTH test (n = 14)				Control test (n = 13)			
	Initial sample	+ 6 h	+ 8 h	+ 10 h	Initial sample	+ 6 h	+ 8 h	+ 10 h
\bar{x}	57.4	63.1	62.2	65.3	60.3	62.1	58.9	60.5
s	4.8	5.9	6.3	7.6	7.5	3.4	5.3	4.6
t	—	2.82**	2.25*	3.30**	—	0.77	0.57	0.09

increasing. All of these changes were statistically significant (***) . No significant changes occurred in the control tests.

6. *The blood sugar concentration* (Table 6) showed a small but significant (*-**) increase after ACTH injection. No obvious changes occurred in the control tests.

Two cows of 25 tested showed a serious allergic reaction, with urticaria, swelling and reddening of visible mucous membranes, and itching, especially around the eyes, nose, vulva and hooves, the reaction appearing immediately after the injection. The symptoms disappeared after an hour or two without further procedures.

DISCUSSION

The variances within the material were rather large. This may be due to the methods used in the blood count. We consider that the inevitable errors in the methods of analysis were reduced as far as possible by counting at least 400 cells on two smears in the differential white count, by using as much as 0.4 mm³ for the total white count, and by repeatedly collecting blood samples both before and after the ACTH injection.

The criterion formerly recommended for interpreting adrenal cortical activity as normal was that, in the individual case, the number of eosinophils in a single blood sample was reduced by more than 50 per cent. For the reasons given above, among others, this criterion is not adequate. This has been shown in earlier studies (*Weirich* 1960; *Ziel* 1963) and is confirmed by our investigations (see s in Table 4). It is evident, however, that an injection of ACTH in dairy cows causes several other changes as well in the white blood picture, and it is obvious that the more

Table 7. Results of ACTH administration, and control test without ACTH injection, for individual cows. + and — denote that the corresponding criterion is/is not fulfilled.

Cow no.	Years of age	ACTH test				Control test			
		1) Decrease of eosino-phils > 50 per cent	2) Increase of neutro-phils > 100 per cent	3) Increase of total white count > 10 per cent	4) Increase of blood sugar > 5 per cent	1) Decrease of eosino-phils > 50 per cent	2) Increase of neutro-phils > 100 per cent	3) Increase of total white count > 10 per cent	4) Increase of blood sugar > 5 per cent
1	3	+	+	+	+	—	—	—	—
2	3	+	+	+	+	—	—	+	—
3	8	+	+	+	+	a)	a)	a)	a)
4	5	+	+	—	+	a)	a)	a)	a)
5	5	—	+	+	+	—	—	—	—
6	5	+	+	+	+	—	—	—	+
7	9	+	+	+	+	—	—	—	—
8	6	+	+	+	—	—	—	—	+
9	5	+	+	+	—	—	—	+	—
10	3	+	+	+	+	—	—	+	—
11	3	+	+	+	+	—	—	+	—
12	4	—	+	+	+	—	+	—	+
13	11	+	+	+	+	—	—	—	—
14	5	+	+	+	a)	—	—	+	+
15	5	—	+	—	+	—	—	+	—

a) not investigated.

Table 8. Comparison between ACTH test and control test in individual cows according to the number of criteria fulfilled for a positive test.

Number of criteria fulfilled	4	3	2	1	0	Number of cows
ACTH test	8	6	1	0	0	15
Control test	0	0	2	7	4	13

factors one can take into account, the more correct will be the estimate.

On the basis of the results here presented we consider that the following method of performing an adrenal cortical test by ACTH injection can be recommended. Two initial blood samples are collected at an interval of $\frac{1}{2}$ —1 hour before an intravenous injection of 200 i.u. of ACTH. Another 2—3 blood samples are drawn between 6 and 10 hours after the injection. All white blood counts should be made by the same person, and at least 400 cells from two smears should be differentially counted unless the eosinophils are counted in a chamber after special staining. The mean value of the two initial samples are compared with the mean value of the post-injection samples. The following criteria should be fulfilled if a test is to be considered completely positive:

- 1) the eosinophils should decrease by more than 50 per cent,
- 2) the neutrophils should increase by more than 100 per cent,
- 3) the total white count should increase by more than 10 per cent,
- 4) the blood sugar content should increase by more than 5 per cent.

As seen in Tables 7 and 8, not all healthy cows in our material fulfilled all of these requirements, but there was a clear difference between the ACTH test and the control test if these criteria were used. The increase of neutrophils seems to be as good an indication of a positive ACTH test as the decrease of eosinophils. The increase of blood sugar content and of total white count are not of the same order of magnitude, but they are valuable aids in the interpretation of the test.

In our view, an adrenal cortical test performed as recommended should be regarded as *positive* if at least three of the above criteria are fulfilled. If only two criteria are fulfilled the test should be repeated. When interpreting doubtful cases the greatest

attention should be paid to changes in the numbers of eosinophils and neutrophils. For a test to be regarded as *negative*, i. e. indicating an adrenal cortical insufficiency, none of the criteria should be fulfilled. If only one criterion is fulfilled, the test should be repeated, at least if the criterion is no. 1) or 2). If these rules of interpretation are followed, one cow (no. 15, Table 7) in the material tested reacted in such a way that the test had to be repeated.

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SUMMARY

We have investigated the effect of intravenous administration of 200 i.u. of ACTH on blood picture and blood sugar content of dairy cows, and have on the basis of our findings worked out a test of the adrenal cortical activity. The mean value of 2 samples collected before the injection is compared with the mean value of 2—3 samples 6—10 hours after ACTH administration. A normal adrenal cortical activity is indicated if at least three of the following criteria are fulfilled:

- 1) the eosinophils should decrease by more than 50 per cent,
- 2) the neutrophils should increase by more than 100 per cent,
- 3) the total white count should increase by more than 10 per cent,
- 4) the blood sugar content should increase by more than 5 per cent.

At an adrenal cortical insufficiency, none of these criteria should be fulfilled.

ZUSAMMENFASSUNG

Test der Nebennierenrinde bei Milchkühen durch ACTH-Belastung.

Die Verfasser haben die Wirkung intravenöser Zuführung von 200 I. E. ACTH auf Blutbild und Blutzuckergehalt bei Milchkühen geprüft und empfehlen den folgenden Test für Kontrolle der Aktivität der Nebennierenrinde. Der Durchschnittswert von 2 Ausgangsproben wird mit dem Durchschnittswert von 2—3 Proben verglichen, welche 6—10 Stunden nach einer ACTH-Injektion genommen sind. Eine normale Aktivität der Nebennierenrinde wird dadurch indiziert, dass wenigstens 3 von den folgenden Kriterien erfüllt werden müssen:

1. Die Anzahl der eosinophilen Leukozyten wird um mehr als 50 % vermindert.
2. Die Anzahl der neutrophen Leukozyten wird um mehr als 100 % vermehrt.
3. Die vollständige Anzahl der weissen Blutkörperchen wird um mehr als 10 % vermehrt.
4. Der Blutzuckergehalt wird mit mehr als 5 % vermehrt.

Wenn keines dieser Kriterien erfüllt wird ist es berechtigt, eine Insuffizienz der Nebennierenrinde anzunehmen.

SAMMANFATTNING

Binjurebarkstest hos mjölkkor genom ACTH-belastning.

Författarna har undersökt effekten av intravenös tillförsel av 200 I. E. ACTH på blodbild och blodsockerhalt hos mjölkkor och på basis av dessa undersökningar rekommenderas följande test för kontroll av binjurebarksaktiviteten. Medelvärdet av 2 utgångsprov jämföres med medelvärdet av 2—3 prov tagna 6—10 timmar efter en ACTH-injektion. En normal binjurebarksaktivitet indikeras av att minst 3 av följande kriterier skall uppfyllas:

- 1) antalet eosinofila leukocyter skall minska med mer än 50 %,
- 2) antalet neutrofila leukocyter skall öka med mer än 100 %,
- 3) totala antalet vita blodkroppar skall öka med mer än 10 %,
- 4) blodsockerhalten skall öka med mer än 5 %.

Uppfylls inget av dessa kriterier finns anledning misstänka en binjurebarksinsufficiens.

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