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From the Department of Reproductive Physiology and Pathology and the Department of Physiology, Veterinary College of Norway, Oslo.

A RAPID RADIOIMMUNOLOGICAL EVALUATION OF THE ANDROSTENONE CONTENT IN BOAR FAT*

By

Øystein Andresen

ANDRESEN, Ø.: *A rapid radioimmunological evaluation of the androstenone content in boar fat.* Acta vet. scand. 1979, *20*, 343—350. — Fat from boars containing androstenone is absorbed to a piece of filter paper. The filter paper is then placed in a tube and a buffer containing antibodies against androstenone is added. Following incubation the filter paper is removed, and by measurement of the residual binding capacity for androstenone in the buffer a relative value for androstenone content in the fat is obtained.

The coefficient of correlation between the results obtained by this rapid method and the ordinary radioimmunoassay was —0.95.

androstenone; radioimmunoassay; fat; boar taint.

Heated fat of some boars gives off an objectionable odour commonly referred to as "boar taint" or "sex odour". According to *Patterson* (1968) the main contributor to this odour is androstenone (5α-androst-16-en-3-one), a steroid produced and secreted by the boar testes (for review see *Gower* 1972).

During recent years radioimmunoassays for the quantification of this steroid have been published (*Andresen* 1974, 1975, *Claus* 1974). These methods are, however, time-consuming and do not lend themselves easily for routine use when a large number of samples are to be evaluated per day. The aim of the work reported upon, was to develop a simple and rapid method which could be practicable for instance for routine evaluation of androstenone content in boar carcasses.

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MATERIALS AND METHODS

Androstenone and tritiated androstenone (specific activity 21.3 Ci/mmol) were gifts from Syntex Research, Palo Alto, California, USA, and dr. J. Rømer, Akademie der Wissenschaften der DDR, Zentralinstitut für Kernforschung, Dresden, DDR, respectively. Other materials: bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, Missouri, USA), dextran T-70 (Pharmacia Fine Chemicals AB, Uppsala, Sweden), Norit "A" (Amend Drug & Chemical Co., Irvington, USA).

Assay buffer

The assay buffer used was 0.05 M Tris/HCl, pH 7.4 with 0.9 % (w/v) NaCl, 0.1 % (w/v) Na-azide and 0.4 % (w/v) bovine serum albumin.

Antibody solution

The antiserum against androstenone (*Andresen* 1974) was diluted 1:20 000 in the assay buffer.

Tracer solution

The organic solvent (benzene) was evaporated in vacuo, and the remaining radioactive androstenone dissolved by adding assay buffer. The final solution contained approx. 7500 CPM ($\approx$18 000 DPM) per 100 µl.

Dextran-charcoal suspension

The dextran-charcoal suspension used for the separation of free and bound steroid consisted of 0.5 % (w/v) dextran T-70 and 2.4 % (w/v) Norit "A" in assay buffer without BSA added.

Samples of fat

Samples of subcutaneous, abdominal fat from carcasses of 28 boars, five gilts and five castrated males have been evaluated. The samples had been stored in the freezer before assay. The samples from the boars were analysed both by the radioimmunoassay previously described (*Andresen* 1975) and by the simplified method, and the results were correlated.

The levels of androstenone in fat from gilts and castrated males have previously been found to be undetectable by ordinary

radioimmunoassay (*Andresen* 1975). These samples were therefore only evaluated by use of the simplified method.

Filter paper for the absorption of fat

The quantity of fat analysed was standardized by saturating a piece of filter paper with fat. Ordinary filter paper ("Ederol", Qualität nr. 15, J. C. Binzer, Hatzfeld, Edeer, Germany), cut into pieces of 10 × 7 mm were used.

Preparation of samples and evaluation of androstenone content

The samples were heated to 37 °C in an incubator, after which an incision was made. A piece of filter paper (10 × 7 mm) was then inserted into the incision. The incision was closed and by applying a slight pressure the filter paper saturated with fat. The filter paper was removed, placed between two other large filter papers and by pressure excess of fat and any possible loose particles were removed.

The piece of filter paper was then placed in an upright position in a disposable glas tube of 3 ml (inner diameter 9 mm). Five hundred µl androstenone-antibodies in a dilution of 1:20 000 in assay buffer was added, and the tubes were placed in a water bath at 20 °C on a mechanical shaker for 1 h. Following this incubation the filter paper was removed by the use of a small pair of tweezers. One hundred µl tracer solution ($\approx$ 7500 CPM radioactive androstenone) was added to the tube which was then agitated, and the incubation continued for 30 min at 20 °C, and then for 2 h in the refrigerator.

Following incubation the tubes were placed in a water bath with cracked ice. After 15 min for temperature equilibration, 100 µl dextran-charcoal suspension was added. The tubes were agitated and returned to the water bath for 15 min. During this period they were agitated once more. Finally the tubes were centrifuged at 2 °C, and 400 µl of the supernatant fluid was transferred to a counting vial, 5 ml of Diluene® (Packard Instrument Company Inc., Downers Grove, Illinois, USA) was added and the radioactivity recorded. The efficiency of the countings was 42 %.

All samples were analysed in duplicate. The standard deviation of duplicate determinations was calculated according to the formula $s = \sqrt{\frac{\Sigma d^2}{2n}}$ where d = difference between duplicate values and n = number of duplicate pairs.

RESULTS

Analyses by the ordinary radioimmunoassay

The content of androstenone in the 28 samples of boar fat varied from 0.30 to 9.16 µg per g fat (Fig. 1). The standard deviation of duplicate determinations was found to be 0.22 µg per g, corresponding to a coefficient of variation of 14.8 %.

Analyses by the simplified method

This method gives the concentration of androstenone in relative values i.e. counts per minute (CPM). The CPM values after

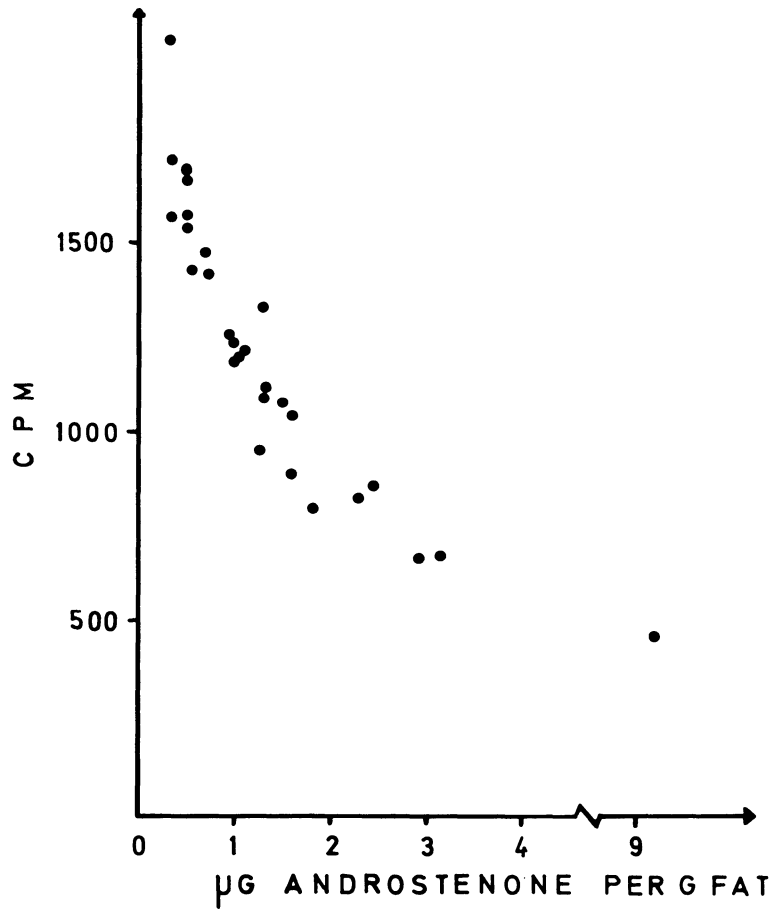

Figure 1. Relationship between the results of analyses of 28 samples of boar fat by ordinary radioimmunoassay (abscissa) and by the simplified method (ordinate).

analyses of the 28 samples of boar fat varied from 2027 to 464 (Fig. 1). The standard deviation of duplicate determinations was 113 CPM, corresponding to a coefficient of variation of 8.9 %. The results of the analyses of the 10 samples of fat from gilts and castrated males resulted in a mean CPM value of 2185 (s = 295 CPM, coefficient of variation = 13.5 %). The mean CPM value of two tubes where the filter paper with fat had been omitted, but otherwise treated as described, was found to be 2543. The mean of two similar tubes where assay buffer instead of antibody solution had been added resulted in 139 CPM.

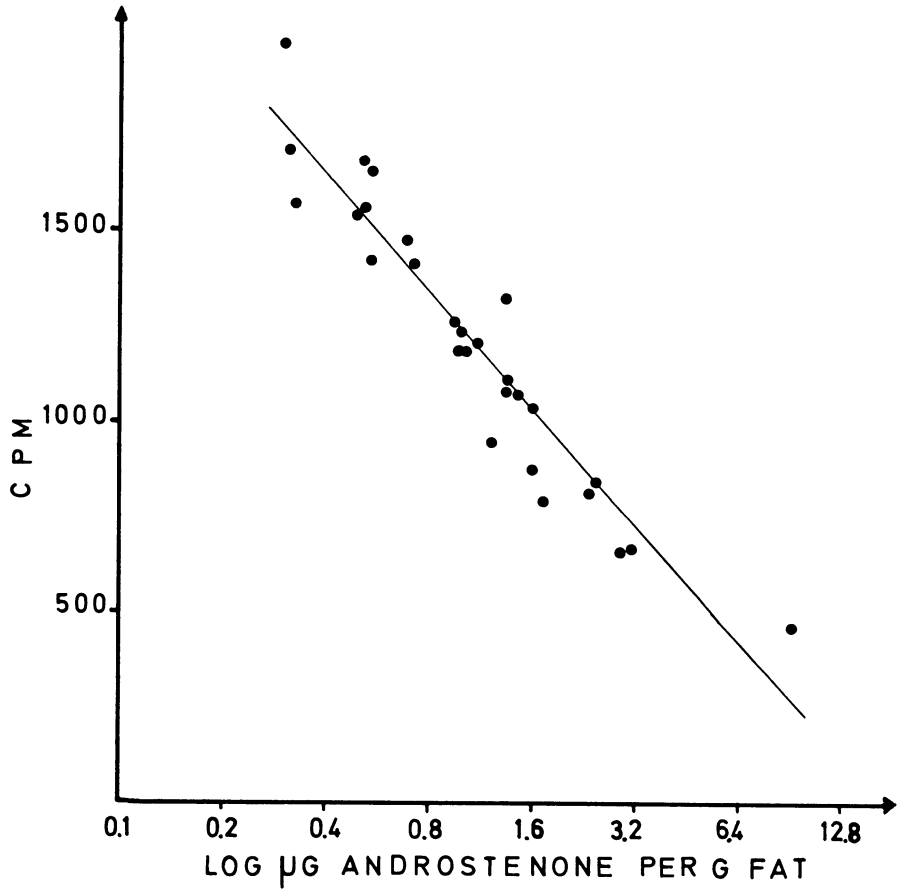

Figure 2. Relationship between the results of analyses of 28 samples of boar fat by ordinary radioimmunoassay (abscissa) and by the simplified method (ordinate). The results on the abscissa is given on a logarithmic scale.

Relationship between the results obtained by the two methods

The relationship between the results obtained by the two methods is illustrated in Figs. 1 and 2. Fig. 1 indicates a curvilinear relationship. Transforming the concentration of androstenone into common logs (Fig. 2) gives a linear relationship with a coefficient of correlation of —0.95. The regression equation was calculated to be

$$y = 1238 - 1035 \log x$$

DISCUSSION

The results of the present study demonstrate that it is possible to obtain an objective estimate of the androstenone content in fat in a very simpel and easy manner. Time-consuming steps as weighing of fat, extraction, purification and evaporation of organic solvents have been omitted. The coefficient of correlation between the results obtained by the previously developed method (*Andresen* 1975) and the present one was, nevertheless found to be very close, and the simplified method therefore seems to give reliable results.

In what way the antibody molecules and the androstenone molecules get into contact has not been studied in detail. It is therefore not known whether androstenone is dissolved from the fat into the buffer or if the antibodies bind to the steroid when androstenone is still in contact with the fat. In any case the polarity of the buffer may be crucial for the binding, and changing the buffers may change the binding.

The simplified method gives relative values of androstenone in CPM. One way to have the androstenone content expressed in µg per g fat would be to include some samples with known concentrations of androstenone. The CPM values obtained from the analyses of these samples could then be used to calculate the regression equation, and the absolute level in unknown samples could be determined by use of this formula.

For routine use further simplifications and automatizations are possible. If large number of samples are to be analysed especially the design of the filter paper will have to be modified in order to facilitate its handling.

As to the capacity of the method one would assume that when fully automatized one technician would be able to analyse at least 100 samples in duplicate per day.

The practical value of the method will have to be established through further work. One problem is to determine which level of androstenone will be accepted by the consumer. *Rhodes* (1971) has suggested 1 μg per g fat as an upper limit, but this may vary with the food habits and the mode of the preparation of pork in the different countries. Another problem will be the possible occurrence of other factors than androstenone that may cause boar taint in boar meat. Androstenone is, however, regarded as being the main factor in the boar taint, and a classification of boar carcasses based on the level of androstenone in fat might make it possible to use boars instead of castrates in the production of pork.

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SAMMENDRAG

En enkel radioimmunologisk metode for bestemmelse av androstenon i fett fra råner.

Fett fra råner som inneholder androstenon ble absorbert til et stykke filtrerpapir. Filtrerpapiret ble plassert i et reagensglass og en tilsatte antistoffer rettet mot androstenon. Etter at antistoff og steroid

hadde bundet seg til hverandre fjernet en filtrerpapiret. Ut fra bestemmelse av resterende bindingskapasitet i antistoffløsningen fikk en et relativt uttrykk for mengde androstenon i fettet.

Korrelasjonskoeffisienten mellom resultatene en oppnådde med denne enkle metoden og den ordinære radioimmunologiske metoden var —0,95.

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Reprints may be requested from: Øystein Andresen, the Department of Reproductive Physiology and Pathology, Veterinary College of Norway, P.O.Box 8146 Dep., Oslo 1, Norway.