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EVALUATION OF A RADIOIMMUNOASSAY TECHNIQUE MEASURING CALCITONIN IN THE BOVINE, OVINE AND PORCINE SPECIES*

By

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FORSLUND, K. and M. STRIDSBERG: *Evaluation of a radioimmunoassay technique measuring calcitonin in the bovine, ovine and porcine species.* Acta vet. scand. 1980, *21*, 155—170. — A heterologous RIA system was set up for measuring bovine, ovine and porcine calcitonin (CT). The system consisted of porcine CT used as standard and for the preparation of an iodinated tracer. The antiserum used was raised against ovine CT. For each analysis was used 25—200 µl blood plasma. Practical detection limit was 0.25 µg of CT per litre of blood plasma.

The parallelism between the dose response curves for the p-CT standard and for the assay of increasing amounts of bovine, ovine and porcine blood plasma showed the suitability of the present assay system to study the CT secretion in these species. Furthermore, the reliability of the method was verified by a clearly recognized CT response to calcium infusion.

calcitonin; RIA; bovine; ovine; porcine; Ca infusion; linearization.

Calcitonin (CT) is a polypeptide hormone produced by the C-cells of the thyroid gland. The hormone is a potent hypocalcemic and hypophosphatemic agent (*Foster et al.* 1972, *Talmage et al.* 1972). In this respect the hormone has been discussed as an etiological factor in parturient paresis in cows.

This condition is an important metabolic disease occurring around parturition in the bovine species. The disease is characterized by decreased blood levels of Ca and inorg. phosphate (P). In

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order to be able to measure possible changes in calcitonin secretion in parturient cows we have evaluated and described a radioimmunoassay (RIA) technique for the measurement of the hormone.

METHODS

Rabbit antiserum to ovine calcitonin was kindly supplied by Dr. Deftos, La Jolla, Calif., USA. The lyophilized antiserum was reconstituted by adding 1 ml of dist. water. The antiserum was then dispensed in 100 µl aliquots and kept frozen until used. Before utilized in the assay system the antiserum was diluted to 1:1000 with the assay buffer (0.05 M phosphate buffer, pH = 7.3, containing 10 % human plasma).

Standard. Porcine calcitonin (p-CT) obtained from Calbiochem AG, Switzerland, was used for the construction of the standard curve. The preparation contained 20 µg p-CT (250 MRC units/mg; *Kumar et al.* 1965) and was diluted in 0.05 M acetic acid (HAc) in such a way that 10 µl of the solution contained 1 µg p-CT. The standard was kept frozen, dispensed in tubes containing 1 µg p-CT each, until used.

Preparation of radiolabelled p-CT

The p-CT used for construction of standard curves was also used for the preparation of radioiodinated p-CT. The p-CT was labelled with ^{125}I using a modification of the chloramine-T-procedure (*Hunter & Greenwood* 1962). Porcine CT used for iodination was diluted with 100 µl phosphate buffer, 0.2 M, pH 7.3. To the tube containing the hormone was added 1 mCi ^{125}I (New England Nuclear NEZ-033H) and 20 µl of a chloramine-T-solution (5 mg/ml in 0.2 M phosphate buffer, pH 7.3). The content of the tube was mixed thoroughly and the reaction was allowed to continue for exactly 30 s after which it was terminated by adding 20 µl of a sodium metabisulfite solution (12.5 mg/ml in 0.2 M phosphate buffer, pH 7.3). When performing the iodination, the reaction tube as well as the reagents were kept in a 0°C ice-bath.

After radioiodination 5 mg of powdered silica (Quso G 32; Bie & Berntsen AS, Denmark), suspended in 1 ml 0.2 M phosphate buffer, pH 7.3, and 50 µl human plasma, was added to the tube containing the labelled hormone. The contents of the tube were carefully mixed (Whirlimixer, Fisons, England) for 60 s. Then the tube was centrifuged at 2000 × g for 5 min and the supernatant containing the free iodine was discarded. The pellet containing the radiolabelled p-CT was then resuspended in 1 ml of an acetic acid-acetone-water mixture (0.1 ml HAc; 2.0 ml acetone; 7.9 ml H_2O), and 12—14 mg Dowex anion exchange resin (Kebo AB, Sweden) was added. Thereafter the content of the tube was carefully mixed for 60 s (Whirlimixer) and then centrifuged at 2000 × g for 5 min.

The supernatant containing the hormone was then further purified on a Sephadex column. About 10 g of Sephadex G-25 fine was

suspended in about 200 ml of distilled water. The Sephadex was allowed to swell on a boiling water bath for 1 h. The column (type K9-60, Pharmacia, Sweden) was packed and kept at 4°C. Flow rate was adjusted to 0.4 ml/min by a peristaltic pump (LKB, Sweden). The elution buffer was a phosphate buffer (0.05 M, pH 7.3) and contained 1 % of human plasma in order to decrease the radiolysis. The supernatant was added to the top of the column and 100 fractions were collected by means of a fraction collector (LKB, Sweden). The radioactivity in each tube was determined in a gamma spectrometer (Elscint Automatic RIA analyzer, Israel). In Fig. 1 is given a typical

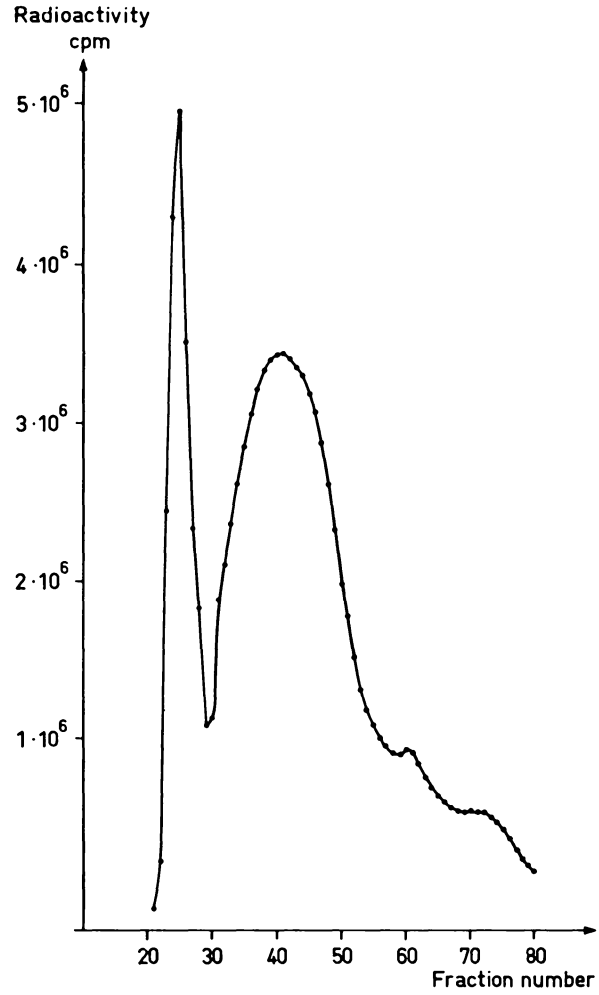

Figure 1. A typical elution curve of ^{125}I labelled porcine calcitonin from a Sephadex column. Each fraction consists of 0.5 ml, flow rate was 0.4 ml/min.

elution-pattern from the Sephadex column. The elution-pattern has been thoroughly examined. The first peak (maximum at fraction 25, Fig. 1) consists of radiolabelled proteins with molecular weights higher than 5000. These proteins bind to the antiserum but not to the same extent as the protein from the second peak. The fractions constituting the second peak (maximum at fraction 40, Fig. 1) contain a radiolabelled protein with a molecular weight around 3000 (molecular weight for p-CT around 3200). Furthermore, these fractions have the highest binding capacity indicating that the second peak is undamaged radiolabelled porcine calcitonin. The small peak at fraction number 60 (Fig. 1) probably consists of calcitonin molecules damaged during the radiolabelling procedure. Since p-CT contains 1 thyrosyl residue per molecule the maximum theoretical specific activity assuming 1 iodine per thyrosyl residue is about 650 mCi/mg. In this study tracer preparations of approx. 65 mCi/mg were obtained. The specific activity was estimated from the radioactivity found in the second fraction and from an assumed mass of 1 μg of CT in this fraction. The specific activity is probably underestimated by this calculation since no correction is made for losses of p-CT.

The suitability of the labelled p-CT, obtained from different parts of the elution curve, as tracer in the RIA system was tested in standard curves. Only radiolabelled hormone fractions from the second peak which resulted in suitable dose response curves were selected for use in the radioimmunoassay system (see Table 1 and Fig. 1).

Table 1. Relative sensitivity of 5 standard curves used for testing the radioiodinated porcine calcitonin as tracer.

Fraction number(s) taken from the elution curve (Fig. 1)	Relative sensitivity[1] of the slopes of resulting calibration lines (%). The most sensitive slope was set to 100 %
25	87 ± 7
33—35	100 ± 5
39—42	100 ± 4
48—50	94 ± 7
60—61	87 ± 5

[1] The slopes (the constant k_2 in Equation 4, Addendum) of the resulting calibration lines were used as an indication of the sensitivity of the assay and hence the quality of the tracer.

Assay procedure

The amount of blood plasma (usually 200 μl and 10 i.u. heparin/ml), 100 μl of the diluted antiserum (1:1000) and the assay buffer to a final volume of 400 μl were added to disposable glass tubes (11 × 55 mm). The CT concentration in the unknown samples was determined

from a standard curve. The curve consisted of the following p-CT concentrations in 6 replicates — 0 (buffer only), 50, 100, 200, 300, 400, 500, 750 and 1000 pg — and was prepared as follows. To each tube was added: 1) the standard amount of p-CT (including amount 0), 2) the same amount of human plasma as the amount of bovine plasma in the unknowns, 3) 100 µl of antiserum (diluted 1:1000) and 4) buffer to a final volume of 400 µl. The contents of the tubes were mixed separately and thereafter incubated at 4°C for 2 days. Then 100 µl (∞ 8000 c.p.m.) of radiolabelled p-CT was added to each of the tubes, and the reaction mixture was incubated at 4°C for another 5 days. Separation of free and antibody bound hormone was achieved by the addition of 1 ml of the antibody to rabbit γ-globulin coupled to a solid phase (DASP, Organon, The Netherlands). The tubes were incubated at room temperature for 2 h during continued agitation and were then centrifuged at 3000 × g for 5 min.

The supernatant containing the free hormone was sucked off, and the antibody bound hormone remaining at the bottom of the tube was resuspended in 1 ml of the assay buffer and then centrifuged again. After removal of the supernatant the radioactivity remaining in the tubes was determined.

The resulting standard curve was fitted to a straight line using linear regression based on the following equation.

$$P = k_1 + k_2 \frac{B_0}{B} \text{ where}$$

P = the dose of unlabelled calcitonin
k_1, k_2 = arbitrary constants
B_0 = radioactivity bound in the absence of unlabelled calcitonin (= activity in 0-tube containing 100 µl of buffer)
B = radioactivity bound to the antibody in the presence of a standard amount of an unknown amount of calcitonin.

For further details on the mathematical evaluation of the equation used, see Addendum to this paper.

Animal experiments

In order to ascertain that the RIA measured CT, a Ca infusion was given to 1 cow and 1 sheep to stimulate the CT release from the thyroid C-cells. Blood samples were collected at —10, 0, 1, 5, 10, 20, 30, 40, 50 min, 1, 1.5 and 3 h after the start of the infusion. The infusion lasted for 45 min in the cow and for 35 min in the ewe. The cow got 10 g Ca^{++} and the ewe 1 g Ca^{++}. The animals showed no adverse side effects in conjunction with the Ca infusion.

The blood samples were collected in Vacutainer tubes (Becton Dickinson, New Jersey, USA) containing heparin (10 i.u./ml) and were kept on ice until the plasma could be separated from

the blood cells, usually within 10 min. Each sample was subdivided into multiple aliquots and kept frozen until assayed.

The blood Ca was determined by an atomic absorption spectrophotometer (Perkin-Elmer 603, Illinois, USA). The precision was < 6 %. The resulting CT and Ca patterns in the cow and the ewe during and after the Ca infusions are presented in Figs. 2 and 3.

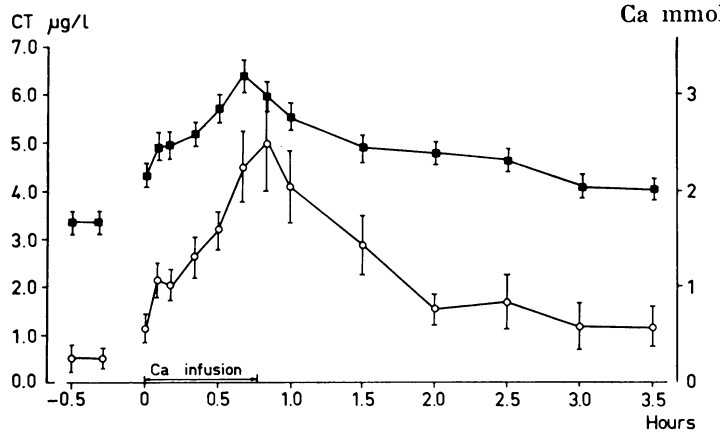

Figure 2. Effect of an intravenous Ca infusion (10 g Ca^{++}) to a cow on the blood plasma levels of Ca (■——■) and calcitonin (○——○). Vertical bars indicate ± 1 s.

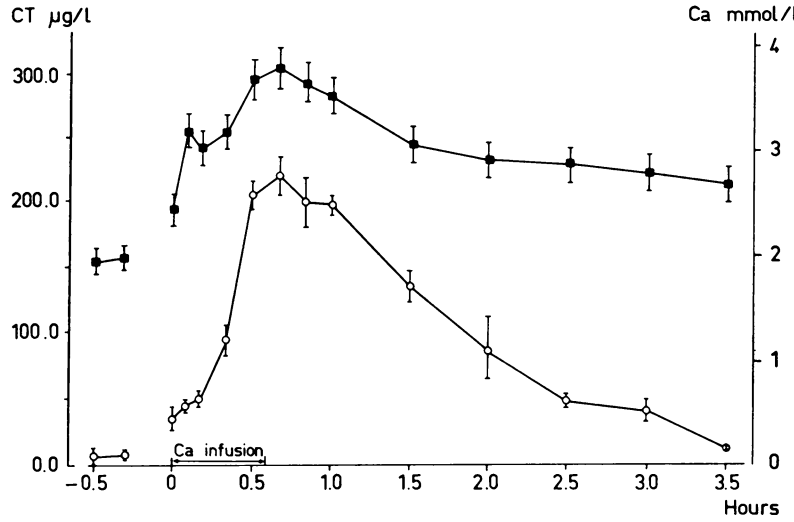

Figure 3. Effect of an intravenous Ca infusion (1 g Ca^{++}) to a ewe on the blood plasma levels of Ca (■——■) and calcitonin (○——○). Vertical bars indicate ± 1 s.

Unstimulated CT levels in plasma from 5 non-pregnant, non-lactating cows and 5 newly delivered cows were measured by the present RIA. Mean blood plasma CT content in these animals was 0.49 μg/l (s = 0.23 μg/l).

To obtain a CT-free plasma to be used as a "blank", 2 heifers were thyroidectomized. Ten days after the operation the animals were sacrificed and bled. Although being thyroidectomized their blood plasma contained CT which did interfere with the assay, and the plasma from these 2 heifers could not be used as a "blank".

Specificity and accuracy

In order to compare the binding kinetics of bovine, ovine and porcine calcitonin in relation to the standard p-CT preparation the following experiment was undertaken. The inhibition given as logit B/B_0 was determined in different amounts of blood plasma (50, 100, 150, 200, 250 and 300 μl) from 1 individual of each of the 3 species. Before the blood samples were obtained from the cow and the sheep, the animals had been stimulated to release CT by an intravenous Ca infusion. Dose response curves were constructed from logit B/B_0 in increasing amounts of plasma from the 3 species and compared with the p-CT standard curve. Parallel curves were obtained. This indicates similar binding kinetics of the antibody for the 3 types of CT (Fig. 4).

Preparations of human and salmon CT as well as heparin were tested, but showed no cross reactivity in the present assay system.

Since no bovine CT preparation of reasonable purity was available, it was not possible to test accuracy in the classical way by recovery experiments. However, the accuracy of the assay can be gained from Figs. 4 and 5, where the results of the assay of increasing plasma volumes from cattle, sheep and swine, with high CT levels, are given.

Sensitivity

Fifty pg of p-CT was found to be significantly different from zero ($P < 0.005$) as read from the standard curve. Since in most cases 200 μl of blood plasma was used for the analyses, the practical detection limit of the assay system was set at 0.25 μg/l.

Human plasma was used in the standard curve to compensate

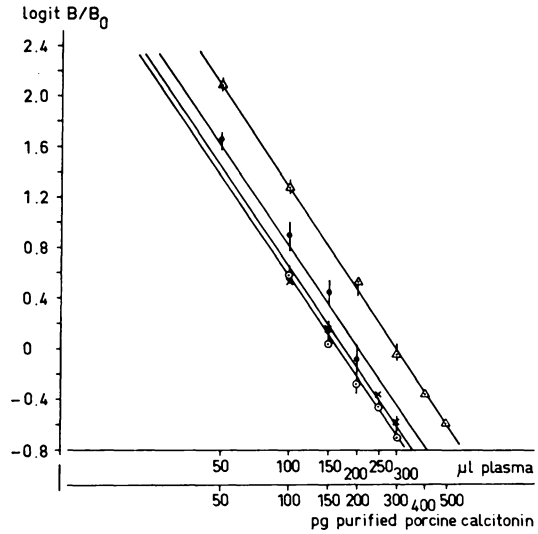

Figure 4. Dose response curves for increasing amounts of blood plasma (µl) from a cow (●), a pig (×), a ewe (⊙) and a porcine standard curve (pg) (△). The curves have been linearized by the logit-log transformation.

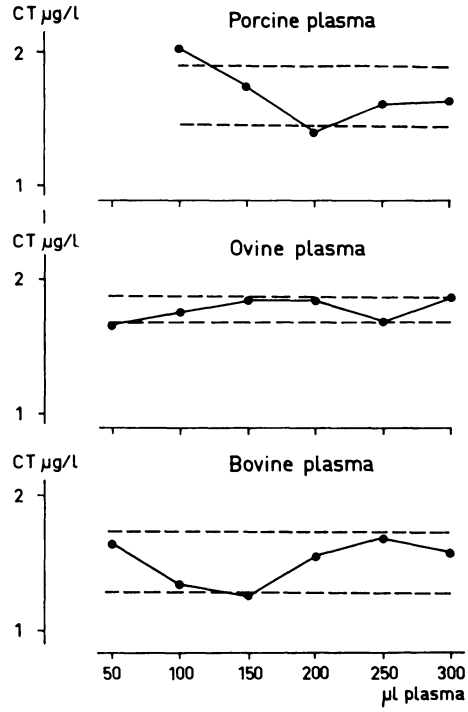

Figure 5. Different amounts of the same blood plasma from a pig, a ewe and a cow analysed and quantified against a porcine standard curve and expressed in µg/l. Dotted lines represent the coefficient of variation calculated from all measurements.

for the possible effects of the blood plasma in the unknown samples on the binding of CT to the antibody. To test the effects of human plasma in the assay system, increasing amounts of human plasma, 25, 100, 200 and 300 µl, were added to the standard amounts of p-CT. No significant effect of the different amounts of human plasma could be detected.

Precision

Within-assay-variation was calculated as the coefficient of variation of measurements of the same plasma pool in 5 different assays. The results are shown in Table 2.

Table 2. Within-assay-variation for the CT measurements given as the coefficient of variation for 9 replicates from 1 plasma pool measured in each of 5 different assays.

Assay no.	Mean concentration of CT (µg/l)	Standard deviation (s)	Coefficient of variation (%)
1	3.3	0.29	8.8
2	2.9	0.45	15.5
3	2.8	0.32	11.4
4	3.5	0.30	8.6
5	2.7	0.23	8.5
Mean	3.0	0.32	10.6

Between-assay-variation was calculated as the coefficient of variation of measurements of the same plasma pool in 5 different assays and was 11.6 %.

Practicability

To test the stability of immunoassayable p-CT in phosphate buffer (0.05 M, pH 7.3), samples containing 500 pg of p-CT were kept at 20°C for 4 days while another set of samples containing 500 pg of p-CT in the above mentioned buffer were simultaneously kept at 4°C. The amount of p-CT measured in the samples kept at 4°C was 450 pg ($s = 140$ pg; $n = 6$) while no p-CT could be detected in the samples kept at 20°C.

To test the stability of bovine CT in the blood plasma samples the following experiment was undertaken: 200 ml of blood was obtained from a cow, which simultaneously with the bleeding was given an intravenous Ca infusion (10 g Ca^{++}). The blood was collected into a flask containing 2000 i.u. of heparin. Five ml of the blood was immediately centrifuged and the plasma was removed and kept frozen until assayed. The remaining part of the blood sample was divided into 2 equal portions. One portion was kept at 0°C in an ice-bath while the other portion was kept at 20°C. Five, 10, 20, 40, 80 min and 5 h after collection of the blood sample a 5 ml sample was obtained from each of the 2 portions. The 5 ml samples were immediately centrifuged and the blood plasma was removed. The plasma was then frozen on dry ice and was kept frozen until being analysed. The samples obtained from the portion of blood kept on ice showed no decrease in the CT concentration during the 5 h test period. The samples from the portion kept at 20°C showed a lowering of the CT concentration already after 20 min of storage. After 5 h of storage only 40 % of the original CT amount was detected.

Rapid freezing and thawing of blood plasma samples did not influence the amount of immunoassayable CT.

DISCUSSION

The present RIA system is of the heterologous type since it utilizes an antiserum to ovine CT and the porcine CT is used for the preparation of the tracer and also used as a standard. The ideal RIA system in an assay for e.g. bovine CT should use bovine CT as the standard. However, when assaying increasing amounts of blood plasma from the cow, the sheep and the pig, it was found that the plasma samples from these species resulted in dose response curves parallel to the dose response curve obtained for the p-CT used as the standard. This strongly indicates the same binding kinetics for the CT in the plasma samples as for the p-CT used for construction of the standard curve. This can be explained by the close similarity in chemical structure existing between bovine, ovine and porcine calcitonin (*Brewer & Ronan* 1969, *Potts et al.* 1970).

Since no bovine or ovine CT of reasonable purity was available to us, we have been unable to develop a factor for the re-

calculation of porcine CT to bovine CT equivalents. However, because parallelism could be demonstrated, it can be stated that although the present radioimmunoassay system probably does not measure an accurate amount of bovine or ovine CT, it will be able to proportionally measure concentration differences of CT on the cow and the ewe. *Deftos et al.* (1972) found a CT level in normal non-lactating cows of 0.162 μg/l (s = 0.012 μg/l) using a bovine CT for quantification. The present system measures the CT level in the blood plasma from normal cows not stimulated with a Ca infusion to 0.49 μg/l (s = 0.23 μg/l). This discrepancy is probably due to the different standard preparations used. However, differences in dietary Ca intake, breed differences, stage of pregnancy, age etc. might also influence the CT level in individual animals.

The labelling technique used here is well established. It is, however, a rather violent method, which usually causes some degree of damage to the protein, e.g. cleavage of the disulfide bonds. The reaction is both time- and temperature-dependent. It was found that exact timing between the addition of chloramine-T and the addition of the sodium metabisulphite (30 s) as well as carrying out the reaction at 0°C were prerequisites in ensuring adequate tracer preparations.

The practical detection limit established for the present assay is partly due to the relatively low specific activity of the radiolabelled p-CT used. Thus, *Deftos et al.* used ovine CT for the preparation of the tracer, and in combination with an antiserum to bovine CT and bovine CT for the preparation of the standard curve they described their assay system to detect less than 0.001 μg/l bovine CT. This high sensitivity is likely to some extent dependent on the higher specific activity reachable with ovine CT, since it contains 3 thyrosyl residues per molecule of hormone. It is very difficult to compare RIA systems using different tracers and standards without testing these against each other in each assay system.

Obviously it is not sufficient to thyroidectomize a cow to obtain CT free plasma. A possible explanation to this could be the presence of C-cells in ectopic thyroid tissues. It is beyond this investigation to verify this assumption.

However, since human CT does not cross-react with bovine, porcine and ovine CT as found here and by *Deftos et al.*, it was

possible to compensate the standard curve for the possible effect of the blood plasma by the addition of human plasma to each of the standard points.

The present finding of rapidly decreasing CT levels in the blood plasma samples stored at room temperature is in agreement with the findings of *Deftos et al.* This makes the procedure for collection of blood samples critical. Thus it is advisable to centrifuge the obtained blood sample and to harvest and freeze the blood plasma as soon as possible upon collection of the sample. This procedure should be completed within 20 min.

The CT response of the cow and ewe to the Ca infusion indicates that the present RIA is measuring CT (Figs. 2 and 3). A directly proportional relationship between elevation in the calcium and the calcitonin concentration was established. The comparatively higher CT level in the ovine plasma has been reported previously by *Garel et al.* (1976). They found that non-pregnant ewes ($n = 10$) had a mean CT content of 9 μg/l ($s = 1$ μg/l). They used a RIA system employing p-CT for tracer and standard, and an antiserum raised against the p-CT. The corresponding unstimulated CT level in the ewe in this investigation was 7 μg/l ($s = 1$ μg/l).

Due to the lack of a suitable bovine calcitonin preparation it has not been possible to prove the accuracy of the present assay system. However, the parallelism between the dose response curves for the p-CT standard and for the assay of increasing amounts of bovine, ovine and porcine blood plasma strongly indicates the suitability of the present assay system to proportionally describe concentration differences in these species.

The development of this specific and rather sensitive radioimmunoassay for bovine, ovine and porcine calcitonin should allow a systematic investigation of the secretion of the hormone in these species.

ADDENDUM

The easiest way to present a standard curve is as a straight line. To achieve such a straight line we applied the law of mole ratio on a mixture of labelled and unlabelled calcitonin. The binding of the hormone to the antiserum was then given by the following equation:

* $$\frac{B}{B_0} = \frac{P^*}{P+P^*} \quad (1)$$

or the inverse

$$\frac{B_0}{B} = \frac{P+P^*}{P^*} = \frac{P}{P^*} + 1 \quad (2)$$

which was first used by *Hales & Randle* (1963).

Thus a straight line is achieved by plotting $\frac{B_0}{B}$ as a function of P. Equations no. 2 can be rewritten to yield

$$P = -P^* + P^* \frac{B_0}{B} \quad (3)$$

or with arbitrary constants

* $$P = k_1 + k_2 \frac{B_0}{B} \quad (4)$$

After employing Equation no. 4 to the values of the standard curve it can, by means of linear regression, be fitted to yield a straight line. This straight line has proven to be the best way for us to evaluate the hormone concentrations in the unknown samples.

Equation no. 1 is often denoted as a rectangular hyperbola forced through origin, and has previously been used by *Bliss* (1970) for evaluating radioimmunoassay data. Furthermore, it has also been used for the derivation of the logit-log transformation (Equation no. 5) proposed by *Rodbard et al.* (1968):

$$\text{logit}\,\frac{B}{B_0} = \ln \frac{B/B_0}{1-B/B_0} = k_3 + k_4 \log P \quad (5)$$

Murphy (1967) used a method called a rectangular hyperbola not forced through origin (Equation no. 6):

$$\frac{B_0}{B} = k_5 + k_6 P \quad (6)$$

Note that Equation no. 6 is mathematically equivalent to Equation no. 4 derived by us.

Since the binding of the antigen to the antiserum is approximately proportional to log P, the linearization-equations using a linear scale for P (Equations nos. 4 and 6) are valid only when equilibrium is reached (*Rodbard et al.* 1969). Therefore, if equilibrium is not reached, it is advisable to use the logit-log transformation (Equation no. 5). This equation can also be useful to detect whether the affinity

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* B = radioactivity bound to the antibody in the presence of a standard amount or an unknown amount of calcitonin.
B_0 = radioactivity bound in the absence of unlabelled calcitonin (= activity in the 0-tube).
P = the dose of the unlabelled calcitonin.
P^* = the dose of the radiolabelled calcitonin (= the tracer).
$k_1, k_2, \ldots, k_8$ = arbitrary constants.

of P to the antibody is different from the affinity of P* to the antibody or not. When this is the case, the slope k_4 is different from —ln 10 = —2.303 (*Rodbard et al.* 1969).

When using Equation no. 2 (which is equivalent to Equation no. 1) it is predicted that the intercept is equal to unity. This is theoretically true. However, we have emperically found the intercept to vary within a range of 0.85 to 1.05.

As mentioned before, the logit-log transformation is derived from Equation no. 1, and therefore it forces the calibration line through a theoretically fixed point. Since this point obviously varies, a modification of the logit-log transformation would be preferable.

Such a modification is proposed in Equation no. 7. The constant k_5 used is the intercept from Equation no. 6. The value of k_5 is also equal to $-\frac{k_1}{k_2}$ derived in Equation no. 4.

Derivation of Equation no. 7:
Start from Equation no. 6

$$\frac{B_0}{B} = k_5 + k_6 P \quad \rightarrow \quad \frac{B_0}{B} - k_5 = k_6 P \quad \rightarrow$$

$$\frac{B_0 - k_5 B}{B} = k_6 P \quad \rightarrow \quad \frac{B}{B_0 - k_5 B} = \frac{1}{k_6 P} \quad \rightarrow$$

$$\frac{B/B_0}{1 - \frac{k_5 B}{B_0}} = \frac{1}{k_6 P}$$

$$\ln \frac{B/B_0}{1 - \frac{k_5 B}{B_0}} = -2.303 \log k_6 - 2.303 \log P$$

$$\ln \frac{B/B_0}{1 - \frac{k_5 B}{B_0}} = k_7 + k_8 \log P \qquad \text{which is Equation (7).}$$

Equation no. 7 gives an even closer fit of the standard curve than the usual logit plot.

$$\ln \frac{B/B_0}{1 - k_5 B/B_0} = k_7 + k_8 \log P \qquad (7)$$

Shaw et al. (1977) pointed out that the logit-log transformation is not an inherently better method than the simpler linearization methods used, e.g. Equations nos. 2, 4 and 5. Therefore and because Equations nos. 5 and 7 involve rather complicated calculations, it is preferable to use Equations nos. 4 or 6 when trying to linearize the standard curve in order to accurately evaluate the hormone content in the unknown samples.

This addendum has briefly analysed the mathematical equivalency

of some well known linearization methods used for radioimmunoassay and has indicated the accuracy and simplicity of the linearization method used in this paper.

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SAMMANFATTNING

Utvärdering av en radioimmunologisk metod (RIA) för att mäta calcitonin hos nötkreatur, får och svin.

En heterolog radioimmunologisk mätmetod för bovint, ovint och porcint calcitonin (CT) i blodplasma har framtagits och utvärderats. I analysmetoden används porcint CT som mätstandard och för preparering av radioaktiv markör. Antiserumet har erhållits efter immunisering av kanin med ovint CT. För varje analys erfordras 25—200 µl blodplasma. Minsta mätbara koncentration av CT är 0,25 µg per liter blodplasma.

Vid bestämning av CT i blod från kor erhölls en CT-koncentration av 0,49 µg per liter blodplasma (s = 0.23). Hos får uppmättes en CT-koncentration av 0,7 µg per liter blodplasma (s = 0.1 µg/l). Genom att analysera CT-koncentrationen i olika mängder av ett och samma blodprov från vardera ko, får och svin samt jämföra de resulterande kurvorna med en standardkurva (renat p-CT) erhölls parallella kurvor, vilket indikerar samma bindningskinetik vad beträffar CT hos dessa tre djurslag.

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