Labour-saving methods for energy-balance experiments with cattle; description of equipment and methods used

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Summary

A number of methods are discussed for reducing the labour required for energy-balance experiments, *e.g.* improved organisation of the work, increased length of the respiration experiment, reduction of the number of analytical determinations, the use of physical methods of gas analysis and the calculation of the results of respiration and balance experiments with an electronic computer. A description is given of the equipment used for measuring gas exchange of cattle.

1. Introduction

A serious drawback of experiments on N, C and energy balances with cattle is the vast amount of work they involve. Most methods used for decreasing this work require expensive instrumentation such as automatic gas-analysis equipment and automatic recorders for punching the data on cards which are then analysed by an electronic computer. While performing balance experiments using two open-circuit respiration chambers we have found that the work can be cut down without this expensive equipment, especially if facilities are available for calculation with an electronic computer. The work was reduced in various ways: –

1. Improved organisation of the work in the experimental period during which faeces and urine are being collected,
2. Increasing the length of the respiration experiments,
3. Reducing the number of analytical determinations,
4. Using low-price physical methods of gas analysis for certain purposes,
5. Recording the results of analysis and other data so that they can be punched on cards without previous correction, rearrangement or copying of figures, and calculation of the final results by means of an electronic computer.

For a better understanding an outline will first be given of the equipment at this laboratory for measuring gas exchange of cattle. Since the schematic description published by VAN ES (1961) some changes have been introduced and a full description is therefore given below.

A survey of the respiration equipment is given in FIG. 1. The two respiration chambers were originally designed by the former director of the laboratory, Prof. Dr. E. BROUWER. Their

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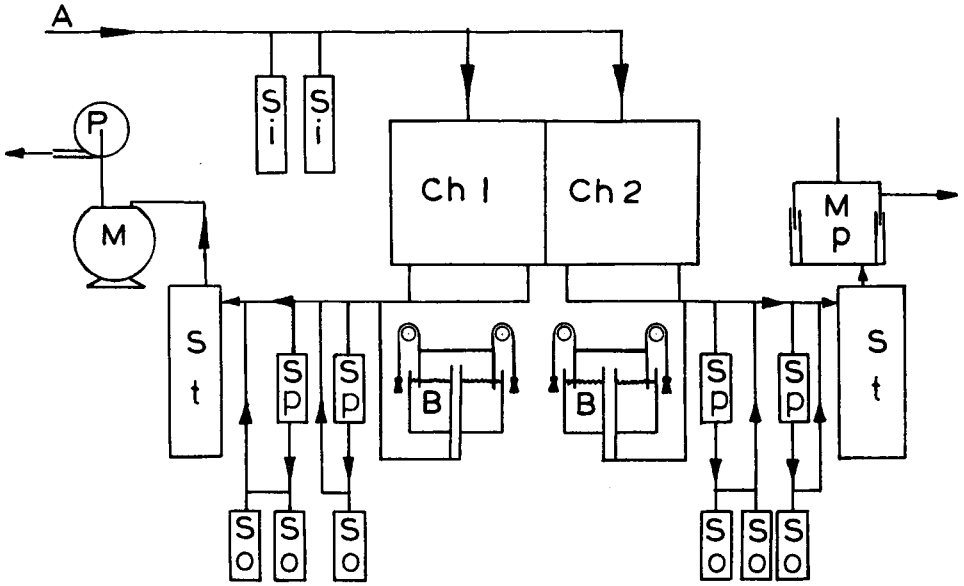
FIG. 1. Diagram of the respiration equipment



A = outside-air inlet; Ch = respiration chamber; Si, So, Sp = collectors of in- and outgoing air samples and sample pumps respectively; St = saturation tower; B = buffer; M = wet-gas meter; P = centrifugal pump; Mp = measuring pump.

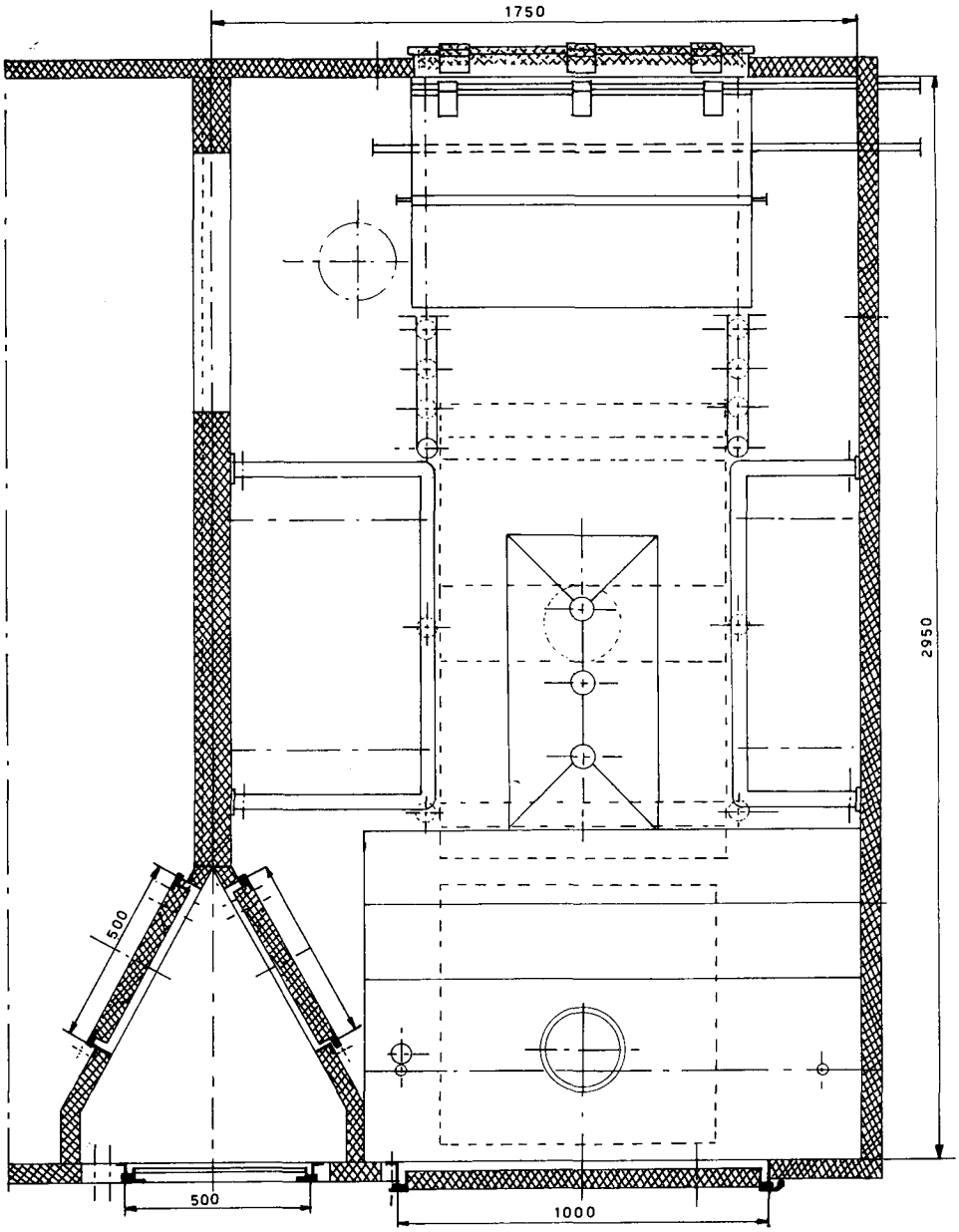
general form (FIG. 2 and 3) differs little from that of KLEIBER's (1935) chambers. The two chambers are mirror images.

Their length is 2.95 m (feeding box 0.60 m, cowstand 1.45—1.85 m, behind the animal 0.90—0.50 m), width 1.65 m and height 2.20—2.38 m. They are made of iron plate (thickness: bottom 5 mm, sides 4 mm and ceiling 3 mm) and insulated by a 50-mm layer of cork covered by a 1-mm aluminium plate.

A window (0.7 x 0.9 m) in the wall between the chambers enables the animals to see each other, and there many small observation windows. The animal enters and leaves the chamber through a large door at the rear (usually after having turned; few animals walk out backwards). This door contains rubber gloves used by the attendant outside for scraping off faeces in the container. There are two airlocks, one (750 l) for entering the chamber and one (250 l) for feeding the animal; both may be used without interfering with the experiment. An air conditioner (1.25 x 0.75 x 0.25 m) with a fan, two cooling (freon expansion) and two heating compartments is suspended from the ceiling. The freon compressor of the air conditioner is in the basement below the chamber. The heating and cooling are respectively controlled by dry- and wet-bulb contact thermometers. If for some reason there is no longer a negative pressure of about 8 mm water inside the chamber, or if its temperature is 5° C too high, alarms fed by an accumulator are set off. One of these is in the house of the head of the experimental farm of the laboratory. Directly the alarm rings a bimetal catch of the lid (0.5 m^2) of a safety airhole in the ceiling is no longer heated. After about 15 min. the catch is cold enough to release the lid which is released by a counterweight from the oil-filled sides of the airhole and allows fresh air to enter the chamber.

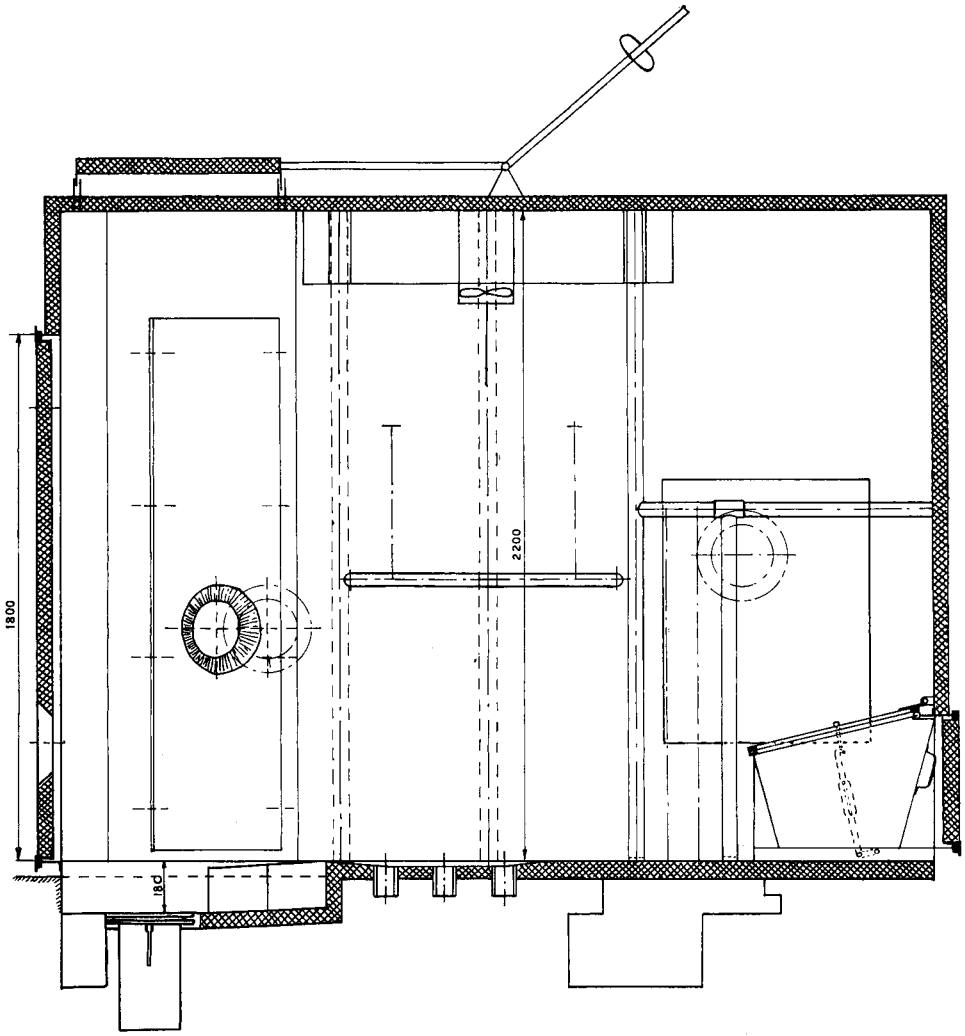
One chamber is ventilated by a gas-measuring pump similar in design to that used by MØLLGAARD (1917), and the other by a centrifugal pump. The air leaving the chamber is first saturated with water vapour in a saturation tower which contains coal over which water is sprayed at 5-minute intervals. The measuring pump is so designed as to deliver the same quantity of air per stroke at all speeds, the number of strokes measuring the volume of the air. The volume of outgoing air

FIG. 2. Plan of the respiration chambers: bottom view



of the other chamber is measured by a large wet-gas meter. Between respiration experiments the gas meter is calibrated by the measuring pump which then draws air through it. In addition, at the start and end of each experimental period of a balance experiment the pump is calibrated at low speed with a small wet-gas meter, itself calibrated by passing through it a volume of air equal to a

FIG. 3. Plan of the respiration chambers: side view



weighed volume of water (VAN ES, 1961). Two buffer spirometers are connected to the pipes of the outgoing air to equalise the pressure fluctuations caused by the air conditioners of the chambers and by the intermittent suction of the measuring pump.

A 16-point resistance-wire recorder measures the temperatures of the measuring pump, the water of the saturation tower connected to it, the gas meter, the chambers (dry and wet bulb), *etc.* It is assumed that the air passing the wet-gas meter is saturated at the temperature of this meter, not at the temperature of the saturation tower connected to it. A barograph checked a few times a day with a mercury barometer supplies information about pressure.

Two diaphragm pumps each deliver 1—2 l per min. of the outgoing air from each chamber from two points near the saturation towers through plastic tubes to the sample containers in the adjoining room. From this gas 0.5 ml per min. is drawn into a glass container and 1.5 or 3 ml

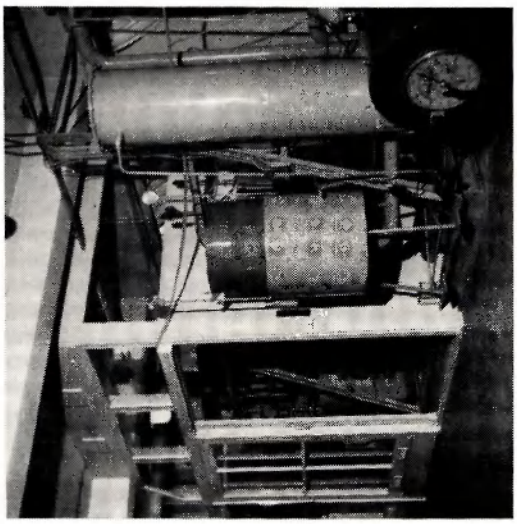


FIG. 5. Measuring pump, buffers, saturation towers, gas meter and centrifugal pump

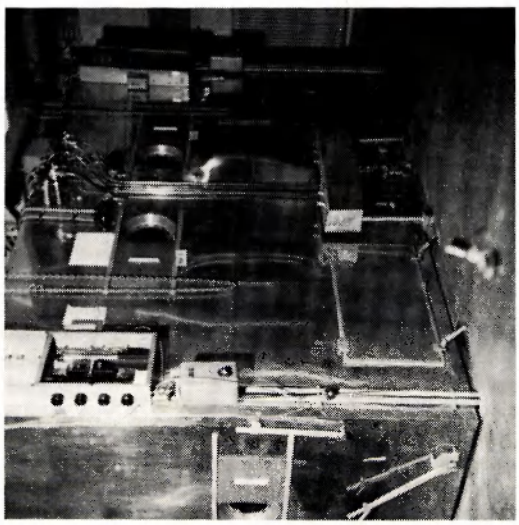


FIG. 4. Respiration chambers

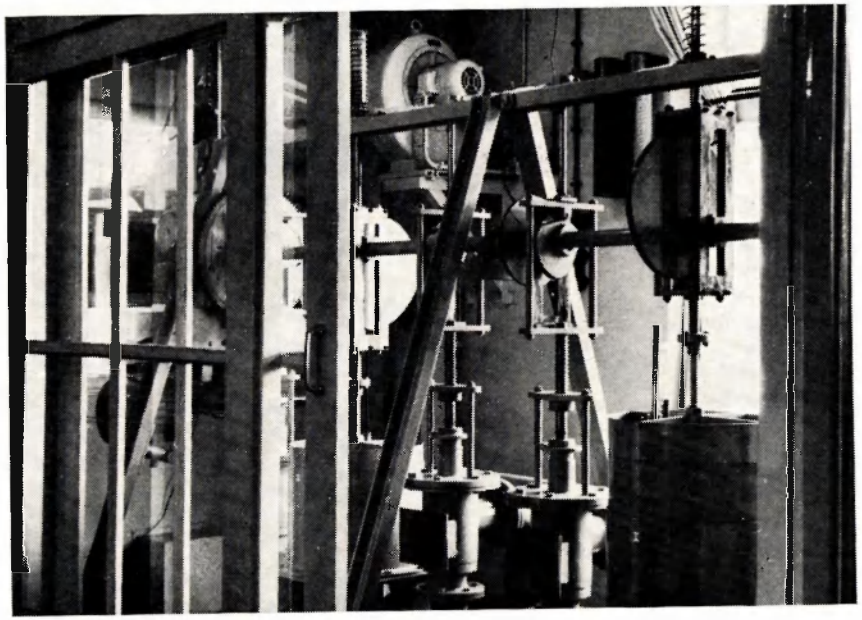


FIG. 6. Measuring pump

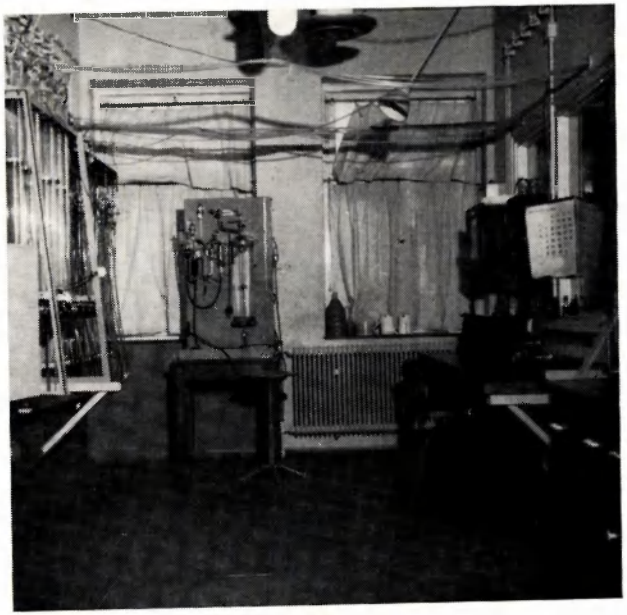


FIG. 7. Gas-analysis room with diaferometer, perspex and glass collectors, Sonden gas-analysis apparatus and recorders

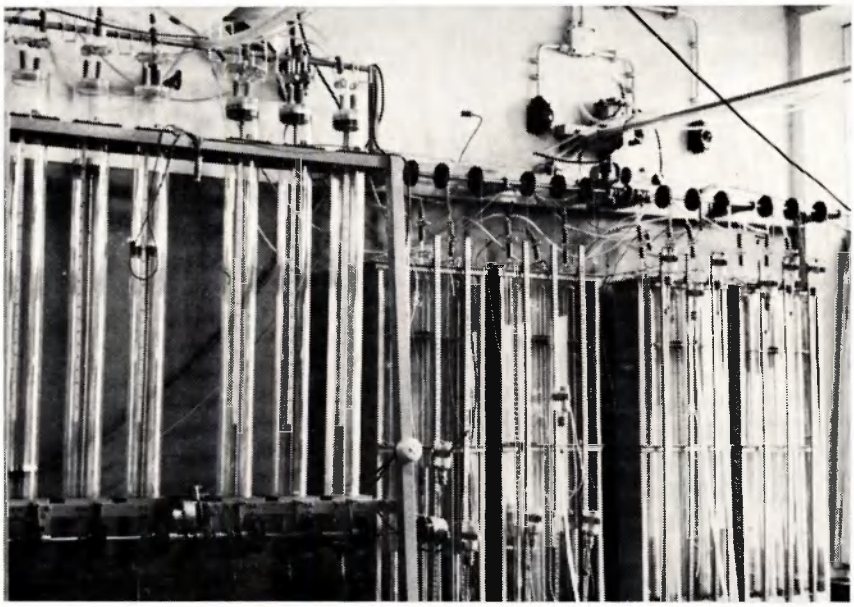


FIG. 8. Perspex and glass collectors of samples of out- and ingoing air

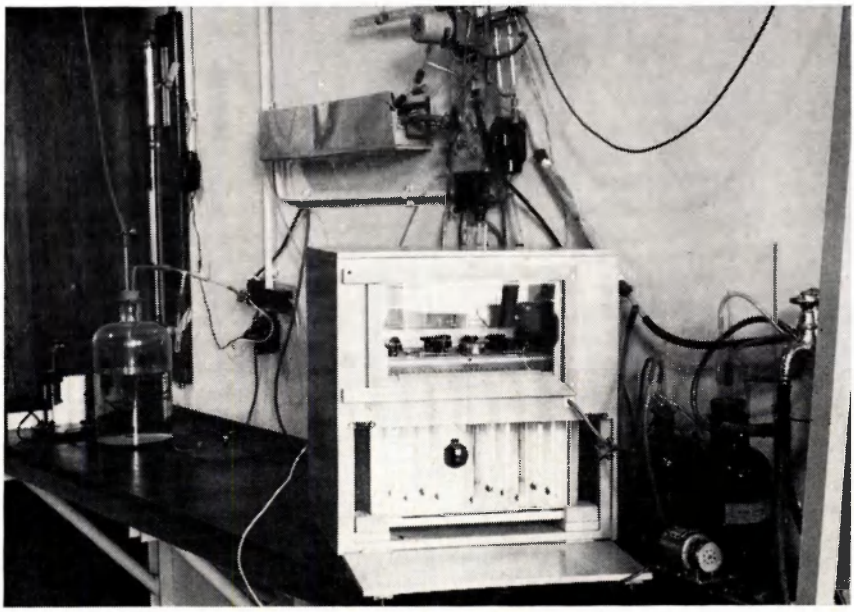
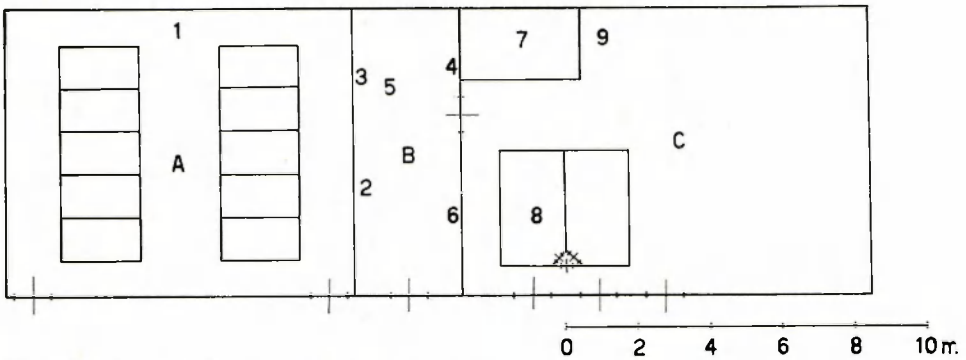


FIG. 9. Diaferometer

FIG. 10. Part of the digestion stall

FIG. 11. Plan of part of the laboratory used for energy-balance experiments



A = digestion stall; B = gas-analysis room; C = respiration room.
1 = scales; 2 = diaferometer; 3 = collectors; 4 = recorders; 5 = Sonden gas-analysis apparatus; 6 = time counters; 7 = measuring pump; 8 = respiration chambers; 9 = buffers, saturation towers, gas meter and centrifugal pump.

into one or two perspex containers, the remainder being returned to the main pipe, thus avoiding the necessity of measuring the volume of this sample gas. As the gas is only in the plastic tubes for a very short time there is no danger of loss of CO_2 through their walls. All containers, the gas-analysis equipment, the measuring pump and the gas meter are in constant-temperature rooms. Each of the glass containers has a capacity of 1.5 l and is completely filled with mercury at the start of the experiment. The mercury flows out of the container *via* an overflow vessel which is lowered by a synchronous motor during the experiment. There are 8 such containers, 4 being used in alternate experiments (one for each diaphragm pump). Four other smaller glass containers (0.8 l) are used for collecting samples of ingoing air. Six 4.5-litre perspex containers are used: two for chamber 1 and one for chamber 2 in one experiment; one for chamber 1 and two for chamber 2 during the next. These containers consist of tubes with a piston on which is placed some mercury. Sample gas is collected in the tube above the mercury by lowering the piston with a synchronous motor.

All gas samples in the glass containers are analysed once for CO_2 and O_2 contents, using a SONDEN apparatus (VAN ES, 1958); one sample per chamber is also analysed for CH_4 content. A diaferometer is used to obtain another determination of the CH_4 content, and for control purpose only, of the CO_2 and O_2 content, in which case a third synchronous motor moves the piston of the perspex containers upward to push the sample to the instrument. The diaferometer is also used for analysing the gas of the chambers for CO_2 and O_2 at the start and end of the respiration experiments. At these times momentary samples of the chamber gas are also collected in small glass containers over mercury, but these are only analysed when there is reason to doubt the results obtained with the diaferometer.
The animals are only in the respiration chambers 2—10 hours before and during the respiration experiments. On other days they are in the nearby digestion stall. Usually the preliminary period of an experiment takes a fortnight and in the experimental period, which also lasts a fortnight, two or three respiration trials are run of 48 hours each.
The lying times of the animals in the chambers and the digestion stall are measured with time counters. Any difference between the two lying times may indicate insufficient training of the animals.

2. Improved organisation of the work in the experimental period

In many balance experiments the same ration will be fed to more than one animal so that sufficient information on the accuracy of the digestibility or metabolizability of the rations may be obtained from the variations between animals. There seems to be little point in also calculating the variations within experiments using data of two or more parts of the experimental period. The collection of this additional data considerably increases the amount of work. Moreover, there is hardly any difference between the variations within experiments and the variation between animals of digestibility and metabolizability (VAN ES, 1961); BROUWER *et al.*, 1964).
It might be objected that if the experimental period is not subdivided, there will be a greater risk of deterioration of the samples of faeces and urine due to the greater length of the collection period. However, deterioration may be prevented by storing the samples at low temperature and with small additions of formalin, acid, HgI_2 *etc.* To prevent the loss of the whole experiment when the animal falls ill ("off feed", *etc.*) near the end of the experimental period, samples collected in the first week may be stored in a separate container and those of the second in another; if the animal remains healthy the contents of both containers may be mixed before analysis.
It is clear that in some cases short experimental periods are to be preferred. The system of *ad libitum* feeding occasionally results in irregular patterns of food intake, in which case it is preferable to obtain results over consecutive short periods. The same applies to experiments in which feeds of varying composition are given, *e.g.* fresh herbage. In experiments with silage, however, we have avoided any possible trouble due to long experimental periods by preparing the day-rations in a special way. All rations of one experiment were placed in large polyethylene bags and weighed on the same day near the start. Before being sealed the bags were twice flushed with nitrogen. Finally all bags were stored outdoors, the temperature during the winter season being between — and + 10° C.
Faeces and urine were usually collected daily in an experimental period, and weighed, mixed and sampled, the sample being added to the samples of the previous days stored in a collective sample vessel. The total daily quantities of faeces and urine of cattle are so large that they cannot be readily stored at a low temperature for many days. In general however it will be possible to store the total quantities of

two days, especially from Saturday morning to Monday morning. Sunday work is expensive and, when it becomes a routine, there is a risk of errors due to the understandable haste of workers to leave the laboratory.

3. Increase of the length of the respiration experiment

At most laboratories respiration experiments last 24 hours and occur during or near the end of the experimental period in which faeces and urine are being collected. Usually 2—6 such experiments are performed in each collection period. Nevertheless, the average results of these respiration trials are used in further calculations and the single results are only used as a means of estimating the accuracy of the data. Hence there is no real reason why respiration experiments should not last 48 hours or even longer, this affecting considerable labour saving. Because of the increase in the variation with an increasing interval between the single or double respiration experiments (VAN ES, 1958; BROUWER *et al.*, 1964), two 48-hour respiration experiments, one near the start and one near the end of the experimental period, are to be preferred to one 96-hour experiment. However, in some special cases there are technical or statistical reasons against respiration trials of over 24 hours. In closed-circuit systems the CO_2 and H_2O absorbers have to be renewed after some time. Two 24-hour experiments are also required to obtain some information on the accuracy of the respiration data when the total respiration period only lasts 48 hours.

In the open-circuit system there is the danger that the stored samples of gas gradually change in composition due to chemical reaction with dirt on the glass or plastic or in the mercury of the containers. With clean containers and mercury we found no changes over periods of three days and more.

The advantage of the 48-hour respiration experiments is that it can be performed from Saturday morning to Monday morning with hardly any additional work during the weekend, especially when the animals are brought into the chambers on Friday afternoon.

4. Reduction of the number of analytical determinations

The number of analytical determinations may be reduced in various ways. Usually, analysis is done in duplicate. To avoid systematic errors both determinations might be done once by two technicians, working independently on different days. The results show whether a third or a fourth determination is needed. An even better method is to take two separate samples. The first sample is analysed once by the first technician, the second once by the other technician; afterwards it should be considered whether further replication is needed. With open-circuit respiration systems it is particularly useful to have two or more independent samples of the air leaving the chamber.

Very often it is possible to plan the experiments in such a way that the same hay, silage, concentrates, *etc.*, are used for more than one animal, making it unnecessary to take one or two samples of each material per animal. In that case the feed should be or be made homogeneous.

When an experimental period is divided into two or more parts it is not usually necessary to analyse the faeces of each part for all components as there is little variation in the composition of the faeces dry matter when a constant amount of food of constant composition is ingested. Dry matter can be determined in the sample of each part and the other constituents in an aliquot mixture of both parts.

5. The use of physical methods of gas analysis

In open-circuit respiration experiments most gas-analysis instruments based on the physical properties of the gases concerned are expensive, partly due to the high degree of accuracy required. Such instruments often only measure the CO_2, CH_4 or O_2 content instead of the content of all three gases. Good instruments based on thermal conductivity such as the Kipp diaferometer compare favourably in both respects; they are low-priced and CO_2 and O_2 contents can be measured simultaneously and the CH_4 content afterwards by combustion of the gas in a silica tube containing a heated nickel-chromium wire (VAN ES, 1958). Their lack of specificity is not important for energy-balance work since interfering gases such as H_2 are rarely present. All such instruments require more maintenance than a good Carpenter, Sonden or Haldane volumetric gas-analysis apparatus. Moreover they should be regularly checked with gases of known composition, *i.e.* with gases analysed with a volumetric apparatus.
Most physical instruments give instantaneous but somewhat less accurate results than a good volumetric apparatus. Obviously therefore a compromise has to be found in selecting the proper instrument depending on required accuracy, technical know-how of the operators, price of instruments and available manpower. Our compromise was to use the Sonden gas-analysis apparatus for determinations which require the highest accuracy and a diaferometer for determinations of less importance and for detecting malfunctioning of the gas-sampling systems. Thus we use a fairly old and not very accurate diaferometer for determining the CO_2 and O_2 content of the gas in the chambers at the start and end of each respiration experiment and for analysis of CH_4, CO_2 and O_2 in the samples collected continuously in the perspex containers. But the CO_2 and O_2 results obtained for the gas of these containers are not used in further calculations, and only serve as a check on the results obtained with the Sonden apparatus for the gas of the glass containers. On days of analysis the diaferometer is checked once with respiration-chamber gas of constant composition stored in a 10-litre bottle over an acidified $CaCl_2$ solution. Results for O_2, CO_2 and CH_4 content of the same samples obtained with diaferometer and Sonden apparatus seldom differ more than 0.02 %, 0.02 % and 0.003 % respectively. The gas in the glass containers is analysed with a Sonden apparatus. Each of two independently taken samples of outgoing air per chamber is analysed once for CO_2 and O_2 content and one of them once for CH_4 content. Two samples of the ingoing air are analysed for CO_2 and O_2 content. The complete analysis with diaferometer and Sonden apparatus takes about half a day for each 48-hour respiration experiment with two animals.

6. Recording of the results; calculation with an electronic computer

A considerable labour saving may be effected by calculating corrections for preservatives, composition of the organic matter of feed and faeces, apparently digested material, digestibility and metabolizability, gas exchange and N, C and energy balances *etc.* with an electronic computer. To avoid copying figures, and thus possibly introducing errors, it is preferable for the technicians to write their results of analyses of feed, faeces and urine on a sheet in such a way that the computer cards can be immediately punched from it. Furthermore, the data of each respiration experiment required for electronic calculation should be written in such an order on each day-sheet that its card can be easily punched. Average temperatures can be estimated from continuous record sheets at a glance with sufficient accuracy provided simple

measures are taken during the experiment to ensure little change in the temperature of the room containing the gas meter or measuring pump.

At this laboratory the results of respiration experiments are calculated immediately after the experimental period. The food has then already been analysed for dry matter and calorific value and the determination of N in urine and of dry matter in faeces is ready a day later. The energy balance can be estimated fairly accurately from this data; the amount of digested energy can be estimated from the gross energy and the digestibility of the dry matter because the digestibility of this substance is usually one unit higher than that of the energy; the energy of the urine may be estimated from the fact that urine often contains 12—17 kcal per g N, the higher figures of the range applying to rations with low protein contents; the energy in methane and the heat expenditure can be calculated from the respiration data. A preliminary estimate of the energy balance during the previous experiment is often of great help in choosing the ration of the next one. The final calculation of the balances is done when all analytical work has been completed.

The card of a respiration experiment contains the following elements: –

```
  NR     M JV BGC TV  T     S     Y    KI KU     ZI    ZU MU   K21Z12
bbb6021112–11 1 08 7473 b196 b214 b004493b610000bb34 14444 b20931b1956 3114 b08b08
```

NR = number (60) of balance experiment, number of cow (2) and respiration days (11, 12).

M = average pressure of gas in measuring pump (mm water).

J = length of the experiment (1 = 24 h, 2 = 48 h).

V = correction for use of feeding device; each time it is used 2 litres CO_2 are lost and 2 litres O_2 gained (litres).

BGC = average barometric pressure corrected to standard conditions (from barograph) (mm Hg × 10).

TV = average temperature at which the air leaving the chambers is saturated with water (in 0.1° C).

T = average temperature of gas in gas meter or in measuring pump (in 0.1° C).

S = number of rotations of gas meter or pump.

Y = volume of gas replaced by one rotation of gas meter or pump (in litres × 10,000).

KI, ZI, KU, ZU and MU = volume % of CO_2 and O_2 in ingoing air and of CO_2, O_2 and CH_4 in outgoing air respectively (× 1000).

K21 and Z12 = difference of volumes of CO_2 and of O_2 respectively in chamber at the start and end of the experiment (in litres; equal to 100 times the difference of the percentages of these gases in the chamber at the start and end).

The computer first calculates the pressure of the water vapour PV by means of a regression equation of PV on TV, TV^2 and TV^3; the volume of the outgoing air ($S \times Y \times 10^{-4}$) is then converted to standard conditions with the aid of T, PV and BGC. The third step is to calculate the volume of the ingoing air, this being done on the assumption that the amounts of ingoing and outgoing dry gas other than CO_2, O_2 and CH_4 are equal. After the differences between the volumes of the in- and outgoing CO_2 and O_2 have been calculated and corrected for the use of feeding device (V) and for gas in the chamber (K21, Z12), and the volume of CH_4 of the outgoing air has been calculated, the heat expenditure (T, kcal) is obtained from:

$$T = 3.866 \times O_2 + 1.200 \times CO_2 - 0.518 \times CH_4$$

It is not yet corrected for urinary CO_2 and N; this is done afterwards during the calculation of the balances. Finally the average O_2 consumption, CO_2 and CH_4 production, methane energy and heat expenditure are calculated when more than one respiration experiment is carried out with the same animal, and also the standard deviation and the coefficient of variation of the last result.

The calculation of the results of 12 48-hour respiration experiments, including preparation of figures for punching, punching and checking of the cards and working with the computer usually takes less than an hour.

For the calculation of the balances and other items, the data of the components of the ration, feed residues, faeces, urine, milk and gas are punched on cards, one or two cards being provided for each of these substances. By means of one or two other cards the computer is informed of the number and type of ration components and whether the experimental period has been split up into two parts, in which case it would receive two cards instead of one for feed residue, faeces and urine. Another card supplies information on the method of correcting intake of metabolizable energy to energy equilibrium.

The ration-component card shows the length of the experiment, total quantity given, % air-dry matter, % dry matter, % crude protein, % crude fat, % crude fibre, % ash and % C, all in dry matter, calorific value in dry matter, and a code number to indicate the type of material (1st + 2nd digits) and numbers of animal (3rd digit) and of experiment (4th and 5th digits). The type of material is coded as follows:

0 or blank = 1st hay	5 = 3rd concentrate	33 = urine
1 = 2nd hay	7 = total intake	34 = milk
2 = silage	6 = feed residue	36 = gas
3 = 1st concentrate	8 = faeces	
4 = 2nd concentrate	9 = digested material	

In the results, composition in organic matter is indicated by a 1 preceding the numerals 0—9 and quantities by a 2.

The feed-residue card shows the same data as the ration-component card, but its total quantity has a negative sign and when minerals were added, it contains their daily quantity.

The faeces card shows the length of the experimental period, total quantity, % dry matter and % crude protein in fresh material, weight of collective samples and of formalin added, % crude fat, % crude fibre, % ash, % C and calorific value, all on a dry-matter basis and code number.

The urine card shows the contents of N, C, CO_2 and calorific value, total quantity, length of experimental period, weights of the collective samples and of the quantities of formalin and sulphuric acid added and the code number.

The milk card shows the total quantity, length of the experimental period, contents of fat, N, C and kcal and code number; the gas card shows the average daily O_2 consumption, CO_2 and CH_4 production, body weight, hours spent lying per 24 h in respiration chamber and in digestion stall and code number.

The various steps of the calculations which lead to the final result (FIG. 12) need little explanation. The figures for composition of faeces and urine in this result are corrected for added preservatives; similar figures of the input cards were uncorrected. The figures below ME.M1, —2 and —3 are the intakes of metabolizable energy

FIG. 12. Record of the electronic computer

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TEST KOE 3  R59

FOOD,DAILY QUANTITY AND COMPOSITION IN DM AND OM
     G     DM     OM     CP     EE     CF     NFE     C     CAL/G  MM
  2100. 89.33 90.44 15.34  3.20 24.04 47.86 44.62 4327.  9.56      359.
  1390. 87.30 98.42 10.49  4.59  3.33 80.01 46.04 4500.  1.58     3359.
   190. 91.71 94.64 55.46  1.54 12.04 25.60 46.80 4749.  5.36     4359.
     -.   .00 99.99   .01   .01   .01 99.96   .01    1.   .01     6359.
      .   .00 92.77 15.53  3.59 15.55 58.09 44.85 4373.  7.22     7359.
                    16.96  3.53 26.58 52.91 49.33 4784.          10359.
                    10.65  4.66  3.38 81.29 46.77 4572.          13359.
                    58.60  1.62 12.72 27.04 49.45 5017.          14359.
                      .01   .01   .01 99.96   .01    1.          16359.
                    16.74  3.87 16.76 62.61 48.34 4714.          17359.
FAECES,DAILY QUANTITY AND COMP. IN DM AND OM
  3156. 19.18 82.80 25.93  4.40 17.21 35.24 45.62 4651. 17.19     8359.
                    31.32  5.32 20.78 42.56 55.10 5616.          18359.
DIGESTED MATERIAL,COMP. IN DM AND OM
      .   .00 95.02 13.19  3.41 15.18 63.23 44.67 4311.  4.97     9359.
                    13.88  3.59 15.97 66.54 47.01 4536.          19359.
DIGESTIBILITY,PCT DIGESTED COMPONENTS IN FOOD-DM AND -OM
      . 81.61 83.59 69.30 77.45 79.65 83.84 81.29 80.44 56.23    30359.
      . 81.61 77.55 10.76  2.78 12.39 51.61 36.46 3518.  4.06    31359.
                    11.60  3.00 13.35 55.62 39.30 3792.          32359.
INTAKE,FAECES,DIGESTED MATERIAL,GRAMS OR KCAL
    DM     OM     CP    EE     CF     NFE     C     KCAL    MM M
 1875.  1696.  287.  60.   450.   897.   837.   8117.  179.    . 20359.
 1213.  1194.  127.  55.    40.   970.   558.   5460.   19.    . 23359.
  174.   164.   96.   2.    20.    44.    81.    827.    9.    . 24359.
    -.     -.    -.   -.     -.     -.     -.      -.    -. 30. 26359.
 3293.  3055.  511. 118.   512.  1913.  1477.  14405.  237.    . 27359.
  605.   501.  157.  26.   104.   213.   276.   2816.  104.    . 28359.
 2688.  2554.  354.  91.   408.  1699.  1200.  11588.  133.    . 29359.
URINE AND MILK,COMPOSITION AND DAILY QUANTITY
   G   PCT N PCT C CO2/FAT CAL/G LCO2/GFAT  GN      GC    KCAL
10887.  .516   .775  .245  74.9       13.   56.1    84.    816.    33359.
     .  .001   .001  .001    .1        .      .0     .       .     34000.
URINE   KCAL/GN   -/GC  -/GE  MILK  KCAL/GN -/GC -/GFAT
          14.52   9.66  5.66          10.00  10.00 10.00 19.23     35359.
   O2   CO2  CH4  HEXP -DIF LYCH LYST    W     W1      W2   W3
1832. 1864. 136.  9184.  64. 11.2 11.4  446.  97.0  .8920  .9178 36359.
CO2C CH4C CH4KCAL -/GE    ME  -/GE  -/DE DCPE/DE
 999.  72. 1285.  8.92  9487. 65.86 81.86 17.44                  37359.
  NBAL   CBAL EBALCN EBALH AV.BAL DIFBAL -/GE  -/ME
    .5    44.   549.   302.   425. -246. -1.71 -2.59            38359.
  ME1  -2      -3   AV.BAL1  -2      -3     ME.M1  -2      -3
97.75 10636. 10336.   4.38   477.   464.   88.98  9681.  9408.   39359.
97.75 10636. 10336.   4.38   477.   464.   89.73  9763.  9488.   39359.
97.75 10636. 10336.   4.38   477.   464.   90.17  9811.  9535.   39359.
```

corrected for body weight and to energy equilibrium, both in three ways. Collecting the data of the cards, punching and checking the cards and calculating the results of 20 balances takes less than 6 hours, the last process only taking half an hour. The results are punched on cards which may be used for reproduction either in the same order or after rearranging by machine or hand. The result cards of a series of experiments in which the same kind of ration was used may be sorted mechanically, using the code numbers in such a way that, for instance, all cards with com-

position of hay, digestibilities, *etc.*, are collected. Each collection of cards may be used as input cards of another programme for the calculation of averages with standard deviations and coefficients of variation. Two more programmes are used for calculating the multiple regression of the corrected maintenance requirement on digestibility, content of crude fibre, *etc.*, assuming constant maintenance requirement of net energy per 500 kg body weight either for all animals, per animal or per two or more successive experiments with the same animal.

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