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Quality of pork in relation to rate of pH change post mortem

by J. Wismer-Pedersen

In recent years the studies of physiological and biochemical factors of importance for the colour and water holding capacity of meat have received growing interest. A part of this interest is undoubtedly due to the growing recognition of the fact that competitive meat products require suitable meat, and the market of fresh meat become more and more discriminative.

Pork seems in particular variable in colour and water holding capacity among the individual animal and deserves therefore special interest. Furthermore, in the veterinary literature cases on pigs have been reported in which the meat has been gray and watery after normal slaughter or by autopsy after instant death on the farm (Ludvigsen, 1954, 1955, Hemmert-Halswick, 1950, Zwijnenberg, 1952, Schönberg, 1957). Excessive feeding with potatoes (Hupka, 1952) or hereditary disposition in connection with stress (Ludvigsen, 1955c, Henry, Romani & Joubert, 1958) have been offered as explanation to the cases.

In this paper some observation on differences in structure, as characterized in the water holding capacity, and colour of pork shall be given. The meat under study comes exclusively from Danish landrace pigs which grew and otherwise behaved entirely normal. The meat was within the quality range pork usually has when offered for consumption in Denmark. Very rarely cases of real "muskeldegeneration" as described by Ludvigsen have been encountered. The pigs were reared on the progeny testing station "Sjælland" and slaughtered at Roskilde bacon factory after electrical stunning. The studies have been concentrated on the loin (m. longissimus dorsi). This muscle is in common experience one of the most variable in colour and texture among the pigs. The colour of the muscle has thus been selected to represent the meat colour in the carcass evaluation as it forms part in the progeny testing of the pigs. (Clausen & Thomsen, 1957).

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Meat structure

Callow (1935, 1937, 1938) was probably the first to study the structure of pork from a technological point of view. He found that the meat could have either an open structure characterized by low electrical resistance and moist appearance by cutting, or a close structure characterized by considerable electrical resistance and dry appearance by cutting. The difference in structure depended on pH and the rate of cooling when the carcass was chilled after slaughter.

In this study attention has been given to the effect pH and meat temperature may have on the structure. This appears especially appropriate as Ludvigsen (1954) has noticed that the pale and watery meat from pigs with "muskeldegeneration" had a pH of about 5.4 when measured 30 minutes after slaughter. A corresponding acidity in the meat at a time when the full body heat still remain may conceivably be of importance to the structure of the meat when ready for use. To establish whether similar low pH values might be encountered in the pigs under study the pH was measured in the loin $\frac{1}{4}$ hour after sticking, i.e. as soon as it is possible under practical condition in the abattoir. The day after slaughter a sample was cut out of the loin at the point where pH was measured to test the water holding capacity. At the same time a new measurement of pH was taken.

Analytical Methods

The pH was measured by direct insertation of the electrodes in the loin right in the back rib. Radiometer pH meter model 24 equipped with glass electrode G213 and kalomel electrode K 4011 were used. The results of the direct measurements have occasionally been checked with pH measurements in samples macerated in 0.005 M sodium iodoacetate.

The water holding capacity was tested by use of a modification of Grau & Hamm's (1953) press method. A pea size, (0.7 g) meat sample was cut out in the middle of the loin and placed in a closed tared test tube until the pressing could take place. The sample was pressed between two filter papers with standardized water content. The filter paper was

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Struers' standard quality kept in a dextricator with saturated KCl solution. The pressing took place in a plexiglass compressorium the plates of which were screwed firmly together for 5 minutes. After the pressing the meat was separated from the filter paper and weighed. The weight loss compared to the initial weight of the sample was used to denote the water holding capacity. The weight in mg. per g. meat will be named the LW number, loose water number. A high number thus denote a low water holding capacity. All the expressed LW numbers are averages of 2 determinations.

The solubility in 0.6 M KCl 12.5 g. meat was mixed in 50 ml cold 0.6 M KCl with an Ultra Tarrax mixer for 1 minute. After 5 minutes 150 ml cold 0.6 M KCl was added to the mixture, which was remixed for $\frac{1}{2}$ minute. The mixture was centrifuged for 20 minutes at 1500 g. With the precipitate the whole operation was repeated. The weight of the final precipitate was recorded, the nitrogen content was determined after Kjeldahl and the percentage nitrogenous material of the meat insoluble in the salt solution was recorded.

The ATPase activity of the meat was estimated essentially as done by Dickens & Salmony (1956) on myosin. Instead of myosin a quantity of homogenized meat devoid of tendons and fatty material was used. The quantity corresponded to 1 mg. dry matter.

The water holding capacity in relation to initial pH

On figure 1 the water holding capacity of the meat tested the day after slaughter is related to the pH as measured $\frac{1}{2}$ hour after the sticking. pH measured at that time will in the following be termed pH_1 . Each point on the figure denote an individual loin. One notice that the pH measurements are spread over a range from about 5.6 to about 6.6. If all the observations are taken together one see a general decrease in water holding capacity with decreasing pH_1 . The correlation coefficient between LW and pH_1 was calculated to + 0.71, which is statistically significant at $P = 0.001$.

For the loins with pH_1 above 6.2 the LW-values are predominantly below 460 while the values for the loins with pH_1 below 6.2 are predominantly above 460. The corresponding correlation coefficients between pH_1 and LW for the loins with pH_1 above and below 6.2 are + 0.38 and + 0.13 respectively. The last mentioned coefficient is far from significance which means that pH_1

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differences in the low pH range have only slight effect on the water holding capacity. The first mentioned coefficient has a statistical probability for significance between $P = 0.10$ and $P = 0.05$ so we may here encounter some relationship between LW and pH_1 .

On figure 2 the LW of the same loins are recorded in relation to the pH of the meat at the time of the LW test. The pH is now in the range between about 5.3 and about 5.6. The coefficient of correlation between LW and the pH is calculated to $+ 0.20$ which is far from statistical significance. There appear thus no intimate relationship between LW and pH in this pH range.

Simultaneous measurements of pH_1 in the loin and gammon (m.adductor) was done in a preliminary experiment. The correlation coefficient between the two muscles was $+0.543$ and found to be statistically significant at $P = 0.001$. The results of the studies of the loin may therefore be applicable to the gammon musculature.

Chemical changes in the meat with low water holding capacities

The differences in the LW numbers might either be due to differences in the water content of the meat or to differences in the water holding capacity. Analysis of meat samples taken together with the samples for the LW test were analysed for dry matter, mineral matter and nitrogen content after Kjeldahl. No differences could be shown with relationship to the LW values. The differences in the LW values will therefore be regarded due to differences in the water holding capacity.

The differences in water holding capacity might be due to partial denaturation of the meat proteins, in particular actomyosin. Denaturation of the actomyosin should manifest itself in decreased solubility in 0.6 M KCl and loss of its enzymatic activity, i.e. its ATPase activity. On figure 3 the solubility of the meat is recorded in relation to the LW number. The experimental material was samples cut out of 13 loins with different LW numbers the day after slaughter. One notice that the meat with LW numbers below 460 contains 20-30 % nitrogenous material insoluble in 0.6 M KCl. The meat with higher LW values has a considerably higher percentage insoluble nitrogenous material arising as high as above 50 % of the total nitrogenous material of the meat. One

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may conclude that the low water holding capacity is connected to an alteration of the meat proteins which have become partially insoluble in 0.6 M KCl. On figure 4 the solubility measurements of the same samples are shown in relation to the corresponding pH_1 . One notices that the low percentages of insoluble material are connected to pH_1 above 6.2. When pH_1 is low the solubility is very variable between the samples of approx. identical pH_1 values. Similar variation was noticed for LW numbers (figure 1).

The measurement of the ATPase activity of samples of loins the day after slaughter did not reveal any close relationship between the enzymatic activity and LW or pH_1 . Low ATPase activity was, however, predominantly associated with pH_1 values below 6.

Low water holding capacity of the loins appears thus associated with structural alterations in the protein towards decreased solubility in 0.6 M KCl. Only sometimes the ATPase activity was also diminished. The impact of the low pH_1 , or the processes which give rise to it, on the meat result first in decreased solubility of the proteins (and increased LW number) and then secondly in diminished ATPase activity.

Continuous record of pH and temperature in the loin

On figure 5 examples are given of the pH and temperature variations which may take place in the loin right from a few minutes after sticking. After the slaughtering operations the carcasses were kept under hanging floor conditions. The measurements were taken on the carcasses from three gilts reared on the experimental farm "Sjælland II" in exactly the same manner as on the progeny testing stations. The pigs were slaughtered at Roskilde bacon factory before the normal slaughtering of the day took place. It was thus possible to do the measurements between normal slaughtering processes like scalding, singeing, etc. The measurements were taken in the loin at the back rib. The measurements continued $6\frac{1}{2}$ hours after slaughter. After the measurements the sides were placed in a chilling room. The next day the loins were cut where the measurements took place and samples taken for measurement of LW number and ATPase activity.

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For gilt 1 the LW number was 421. The corresponding pH fall (1 on the figure) may therefore be regarded as harmless to the meat structure. The colour of the cut surface of the loin was judged excellent (score $2\frac{1}{2}$ on the scale p). For gilt 2 and 3 the LW numbers were 482 and 494 respectively. The corresponding pH falls (2 and 3 on the figure) may therefore be regarded harmful to the meat structure, 3 more so than 2. The meat colour was judged pale and the meat had a wet appearance (score 1 and $\frac{1}{2}$ respectively).

The ATPase activities were 15.3, 14.3, and 9.7 for gilt 1, 2, and 3 respectively. The units of activity is μ g P liberated on incubation in 15 minutes at 30°C. It appears that the pH fall corresponding to 3 has caused changes in the meat proteins so the enzymatic activity has decreased. For gilt 2 the pH fall has decreased the water holding capacity but insignificantly depressed the ATPase activity compared to the measurements for gilt 1. This result is in line with the notion that the differences in pH variations affect the water holding capacity to a greater extent than the enzymatic activity.

In order to get a starting point for consideration of the pH fall in the carcass it was attempted to measure the pH while the pig was still alive.

About a fortnight before slaughter the gilts were subjected to trichlorethylene anaesthesia and a sample of the loin muscle removed. The sample was instantaneously macerated in iodoacetate and pH measured. The pH was 6.75, 6.69, and 6.67 for gilt 1, 2, and 3 respectively. The pH were thus all relatively high and reflect strikingly the drastic pH changes which take place in the course of slaughter. Under the stress which the anaesthesia and biopsy undoubtedly imposed on the animals the gilts 2 and 3 show lower pH values compared to gilt 1. When this observation is related to the pH falls after slaughter it appears likely that the initial step of the pH fall at slaughter starts before death under influence of the stress the animal is subjected to.

The cause to the difference in rate of pH fall

Analysis of the meat

On figure 6 is shown the relation between pH_1 and the content of lactic acid in the meat at the time of the pH_1 measurement. The content of lactic acid was determined after Barker & Summerson (1941). Each point on the figure represents an individual loin. The coefficient of correlation between pH_1 and the lactic acid concentration was worked out to be + 0.86 and found to be statistically significant at $P = 0.001$. The fall of pH must thus be regarded due to formation of lactic acid. Analysis of the content of glycogen, reducing sugars, and lactic acid in some of the loins showed a lower glycogen content and higher content of lactic acid and reducing sugars in the loins with low pH_1 compared to the loins with high pH_1 . The formation of lactic acid may therefore be ascribed to accelerated glycolysis. The analysed samples were taken when pH_1 was measured and frozen with carbon dioxide snow until determination could take place. The methods of analysis were the same as described previously (Wisner-Pedersen, 1957). In the same way the content of total, inorganic and "7 minutes' phosphate" in the meat was estimated. "7 minutes' phosphate" is usually used as an estimate of the ATP content. The methods of analysis were ^{as} given by Bendall & Bate-Smith (1947). The results did not reveal any differences of interest in the interpretation of pH_1 . The content of $P_{7 \text{ min.}}$ was on an average 0.21 mg./g. meat when the pH_1 was below 6.2 and 0.25 mg./g. meat when pH_1 was above 6.2. The results thus suggest that a violent splitting of ATP take place in the meat with low pH_1 if one consider that $1\frac{1}{2}$ -2 mol. ATP are reformed for each mol. of lactic acid formed (Lipmann, 1941).

Determination of magnesium and calcium in the meat did not show any difference in concentration in relation to pH_1 . Magnesium was determined gravimetrically and calcium titrated with permanganate after precipitation with oxalate.

The concentration of potassium was on an average a little lower in the meat with low pH_1 . There was, however, considerable variation between the loins

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with similar pH_1 so low pH_1 was not allways connected to low potassium content. The method of analysis was essentially as described by Ludvigsen (1954). The lower potassium content may be ascribed to leaking of potassium from the cells. The extravasation of potassium was also noted by Ludvigsen (1954) in his examination of the meat from pigs with "muskeldegeneration". It is probably due to formation of lactic acid in the cells before death (Henry, Romani & Joubert, 1958).

In an attempt to study the mechanism of the accelerated lactic acid formation the phosphorylase activity was estimated in 6 tenderloins (m.psoas) with different pH_1 . The estimation was made essentially as described by Sutherland & Cori (1951). The meat was macerated as soon as possible after measurement of pH_1 . The maceration was done in a cold 0.04 M phosphate buffer, pH 7.4, containing 0.001 M versene and 0.02 M sodium fluoride. The macerate was incubated for 30 minutes at $37^{\circ}C$ with an amount of glycogen corresponding to 1 % of the weight of the meat. On figure 7 the percentage of the added glycogen decomposed during the incubation is shown in relation to the pH_1 of the meat for each of the 6 tenderloins. The results suggest that the phosphorylase activity is increased in the meat with low pH_1 . The amount of lactic acid and reducing sugar formed had no quantitative relationship to the decomposed amount of glycogen. The discrepancy is probably due to disorganization of the cell structure on the maceration. The increased phosphorylase could possibly be a consequence of adrenaline released from the adrenals. Estimation of adrenaline content in blood samples taken at the sticking operation failed, however, to show any relation to the pH_1 values of the loins. The method of estimation was essentially as described by Weil-Malherbe & Bone (1953).

Histological examination

Samples of loins with different pH_1 have kindly been examined histologically by Erna Christensen, M.D., Rigshospitalet. No systematic difference in the appearance of the muscle fibres and the cell structure has so far been encountered between the meat with high and low pH_1 .

Observations on the pigs

The rapid production of lactic acid appears to be a consequence of increased demand of energy in the cell which exceeds what can be achieved through the aerobic metabolism. This demand may be a further consequence of a stress on the animal. The idea about stress is in line with common experience in the trade that more pigs with pale and watery meat show up when the pigs have a strenuous transport to the abattoir, especially in hot weather. It may also be shown that the amount of fighting and turmoil among the pigs in the pens before slaughter have a pronounced influence on the occurrence of meat with low pH_1 .

On figure 8 observations on the behaviour of pigs in the pens at the abattoir before slaughter are related to variation in the frequency of pigs with loins having pH_1 below 6.2. The figure also show the frequency in relation to the time the pigs are detained in the pens. The pigs under observation were 850 pigs from the progeny testing stations delivered to the abattoirs in the months June, July and August 1957 after less than 13 km transport by lorry. The pigs were delivered to the abattoirs in lots of 20-30 pigs each week. The lots were watched for the length of time in the pen before slaughter and the degree of turmoil. The lots were then classified in accordance to turmoil and detainment and the pct. of pigs giving $pH_1 < 6.2$ were averaged among the pigs noted. The decrease or increase in occurrence of low pH_1 in percentage of the average frequency is given below and above the 0 line. When the pigs were slaughtered within 20 minutes after delivery the frequency was 2 % higher than average. If there were allowed a detainment of 20-59 minutes before slaughter the frequency was 7 % below average. For longer detainments the frequency rose above the average as the pigs would commence fighting. Fighting obviously has considerable influence on the frequency. If the pigs appeared calm in the pen the corresponding loins showed a reduced frequency of about 33 %. Calmness was, however, recorded only when the pigs were less than 1 hour in the pens. If, however, heavy turmoil occurred the frequency of low pH_1 was above average irrespectively of the length of detainment.

In order to learn more about the effect of turmoil on the pigs we have looked into the possible relationship between the appearance of the carcass

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with respect to bruises and scratches and the pH_1 of the loin. The carcasses under study were of pigs delivered from the progeny testing stations "Sjælland" and "Jylland". Bruises mainly arise from pushes or blows delivered the animals by men, and perhaps fellow pigs, during driving of the pigs from lorry to pen or from pen to sticking floor. All bruises of a size bigger than 5 cm^2 are recorded. Scratches mainly arise from the teeth when the pigs attempt to bite each other. Sometimes also from the hoofs when a pig attempts to climb on another pig.

On figure 9 increase or decrease of loins with pH_1 below 6.2 is given in percentage of the average frequency of the stations. On the columns are given the number of observations. One notices that the frequency is increased for the pigs with bruises especially when bruises and scratches are combined. When the carcasses have no bruises or scratches the frequency is reduced. When the carcasses have only scratches the frequency is not substantially affected, for "Jylland" even decreased compared to the average frequency. The effects of the impacts on the animals giving rise to bruises are especially notable for the stations "Jylland". This is interesting in view of the fact that the average level of frequency was somewhat lower for "Jylland" than for "Sjælland". A number of factors possibly act towards increasing the frequency. When the impact of other factors are slight the effect of the factors under study will be more spectacular.

These results suggest that the processes in the live pig staging the rapid pH fall in the loin are to a considerable extent governed by fright and shock rather than the mere exercise of turmoil and driving. A blow given a pig probably appears more frightful to it than an attempted bite by a fellow pig. When the pigs are excited as a consequence of, or occasion for, turmoil they will be correspondingly difficult to drive and bruises may result.

These observations are somewhat in line with observations made by Howard and Lawrie (1956) on steers. "Excitability" rather than exercise brought about glycolysis in the muscles. For the steers the glycolysis resulted in depleted glycogen reserves while for the pigs under our study the glycolysis manifested itself in the rapid pH fall post mortem.

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As far as seasonal variations are concerned the frequency of pigs giving loins with pH_1 below 6.2 are increased in the summer time, especially in hot months.

On figure 10 variations in the frequency of pigs with pH_1 below 6.2 in the loin are recorded for the month June to December 1957 around the average frequency. June and July were hot months.

The same variation in frequency has been found in cases of real "muskel-degeneration" and has been attributed to decreased activity of the thyroid gland (Ludvigsen, 1954). An explanation of the possible role of the thyroid in this connection could be the following. It has been well established that the content of PBI (protein-bound-iodine) in the blood of pigs is lowest in the hot summer months (Sørensen, 1957). Regulation of the oxygen consumption may be the primary effect of the thyroid hormone (Cori, 1956). A low concentration in the blood may effectuate decreased oxygen consumption in the muscle cells. The increased energy demand in the cells when the animal is under stress conditions may sooner switch the metabolism into the more expensive anaerobic phase when the oxygen supply is decreased. Such conditions may well contribute to initiation of the rapid post mortem pH fall.

Effect of the difference in structure
on the quality of fresh meat

It might be of interest to evaluate to which extent the consumer may perceive difference in the quality of pork with different water holding capacity. To gather information on this point the colour was appraised in the fresh cut of the loin at the last rib the day after slaughter. The taste and texture was evaluated in fried chops cut from the back part of the loin.

Analytical Methods

The meat colour. The appraisal was done by placing the meat in one of seven colour classes to which there was assigned colour scores from 0.5 to 5 (Clausen & Thomsen, 1957). The classes were the following:

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	<u>Colour score</u>	<u>Description of the meat colour</u>
1)	0.5	grey, the same colour as boiled meat, very wet appearance.
2)	1.0	very pale pinkish, wet appearance
3)	1.5	pale pink, slightly wet appearance
4)	2.0	slightly paler than desirable
5)	2.5-3.0	desirable red colour
6)	3.5-4.0	slightly darker than desirable
7)	4.5-5.0	very dark

The haematin content of the meat was determined as done by Lawrie (1950).
As standard an alkaline solution of haemine (Hoffman la Roche) was used.

Colour

The impression one gets of meat colour will partly depend on the pigment concentration, partly on the meat structure. On figure 11 the average colour of the loin from a number of pigs is shown in accordance to meat structure, denoted by the LW number, and the haematin concentration which is taken as a measure of the pigment concentration. The examined loins were first grouped after haematin content, then each group was split in two subgroups of loins with LW number below and above 460 respectively. For each subgroup the average colour score was calculated and illustrated with the length of the column. All the loins examined had colour scores between 1 and 3. One notices that the meat with LW numbers below 460 have obtained colour scores about 2.5-3.0 irrespectively of the pigment concentration. The average score rose from 2.5 to 3.0 with increasing pigment concentration. For the meat with LW numbers above 460 the average colour score for the two groups with lowest haematin concentration was about 1.5, i.e. the meat had a pale colour. In the groups with higher pigment concentrations the colour was on an average better, but the results showed considerable variations.

In general one may conclude that the meat with normal water holding capacity has desirable colour even at the lower pigment concentration. When the structure is changed as illustrated in LW numbers above 460 the meat has pale colour for the most usual pigment concentrations. With higher pigment

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concentrations the meat may, however, have desirable colour in spite of the adverse structure.

Taste and Texture

On figures 12 and 13 the scores for taste and texture of 23 fried pork chops are given in relation to the LW number of the raw meat. The scores are given by a trained taste panel with 8 members. For the scoring a scale of 10 marks was used. 8 denoted "good" and 6 "slight offflavour" for the taste and "slightly tough or loose" for the texture. Each recorded score is the average of the panel for each chop.

The distributions of the scores show that the meat is absolutely acceptable for consumption over the entire LW interval (400-550). There were, however, a slight decrease in the scores for taste as well as texture as the LW numbers increase. There appeared to be no leap in these quality characteristics at LW numbers around 460.

Summary

Some observations on the meat quality in the loin from Danish landrace pigs have been made. The pigs under study were reared and fed under uniform conditions. For a part of these pigs a very rapid fall in pH post mortem was recorded. In an hour the total pH fall had almost occurred. The meat with this rapid pH fall had decreased water holding capacity and the proteins had lost a part of their solubility in 0.6 M KCl. The rapid pH fall was caused by accelerated glycolysis. The frequency of pigs giving rapid pH fall appeared related to the amount of stress the animals were exposed to before slaughter. Fright appeared more important than mere exercise. The effect of the decreased water holding capacity on the general quality of the meat was evaluated. The raw meat colour was in general paler while taste and texture of the fried chops were not essentially affected.

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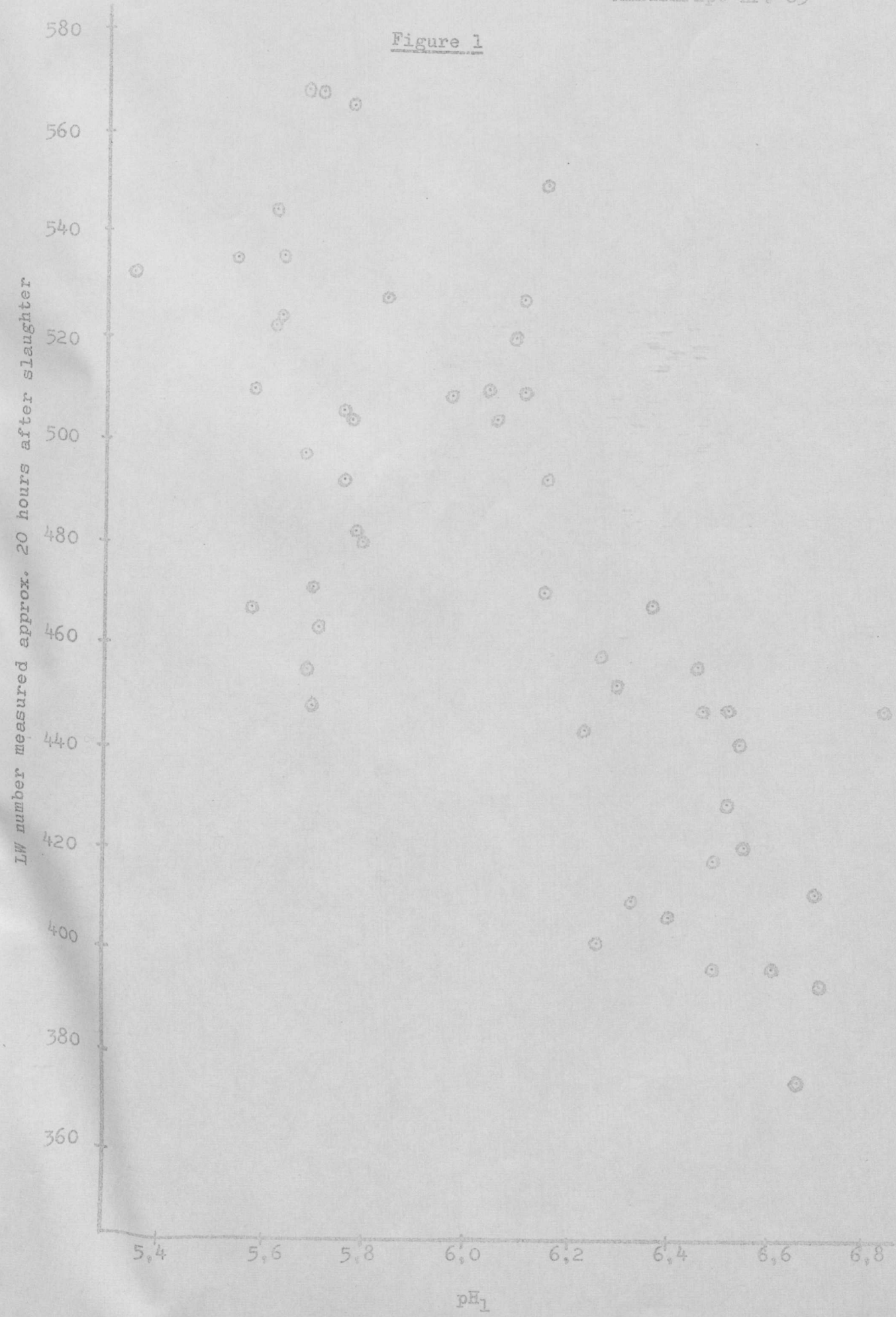
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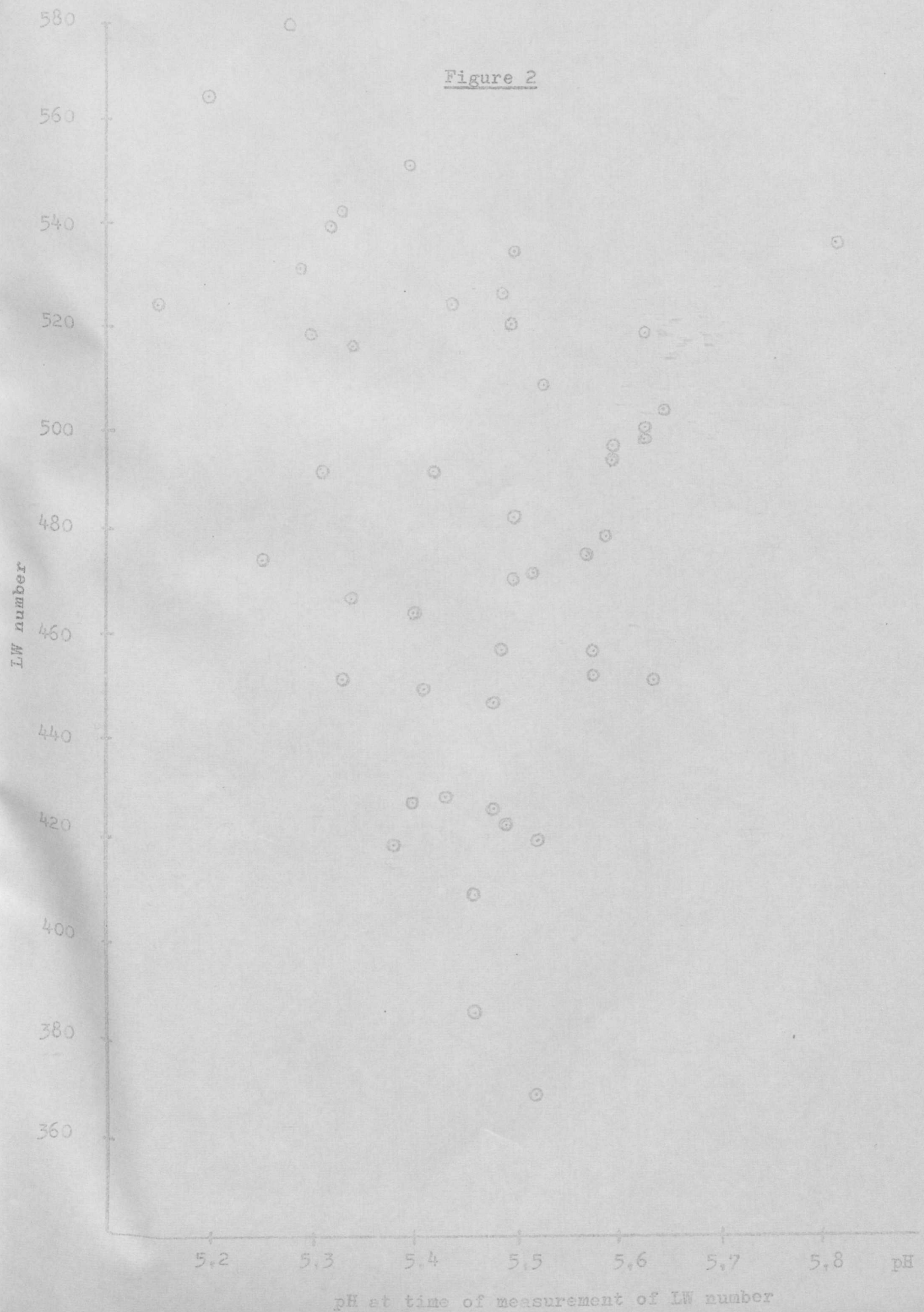
Figure 1



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Figure 2

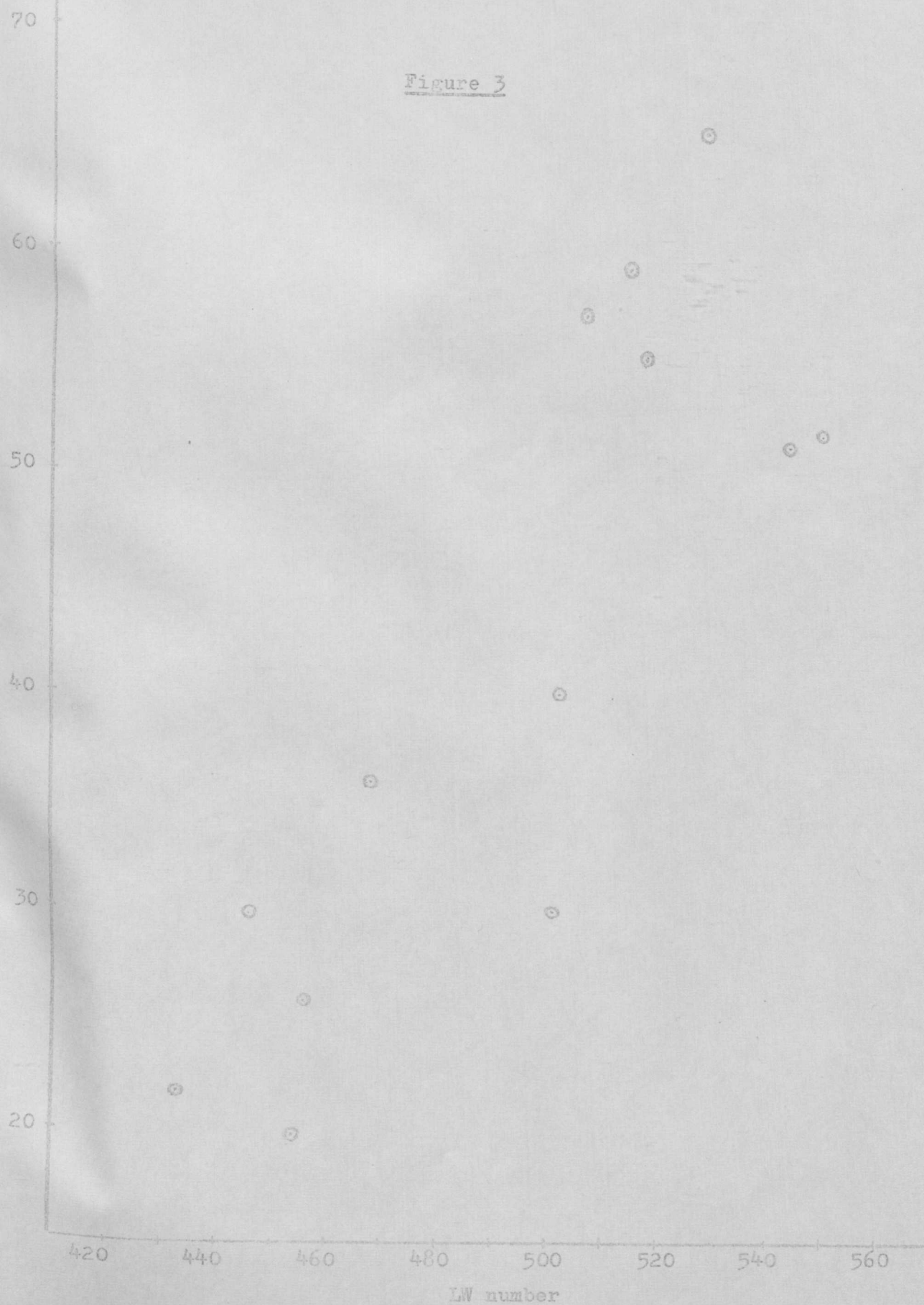


SLAGTERIERNES FORSKNINGSinSTITUT
25. august 1958

MUSKELDEGENERATION
Manuskript nr. 83

percentage meat N
insol. in 0,6 M KCl

Figure 3

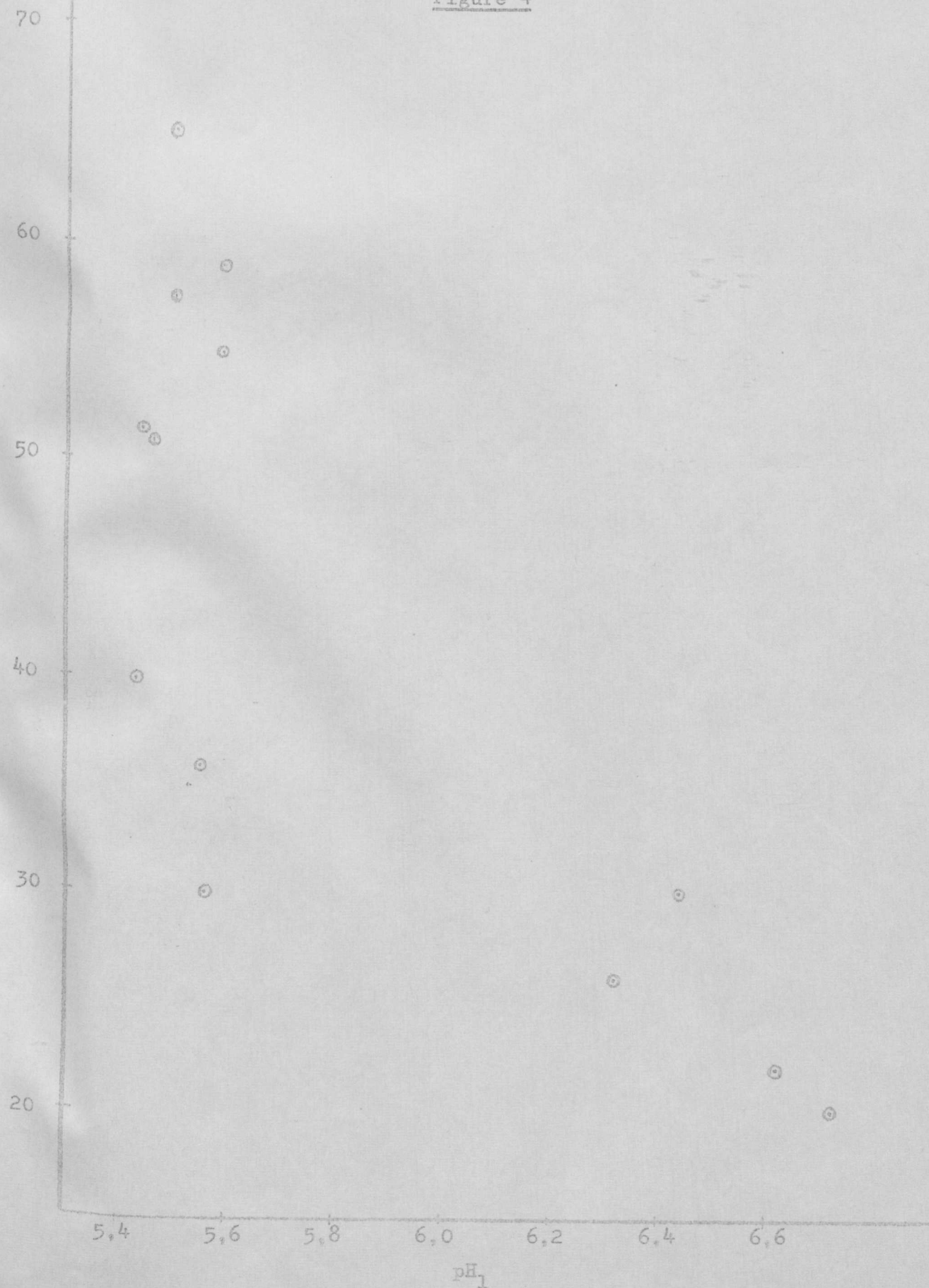


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MUSKELDEGENERATION
Manuskript nr. 83

Percentage of meat N
sol. in 0,6 M KCl

Figure 4



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Manuskript nr. 83

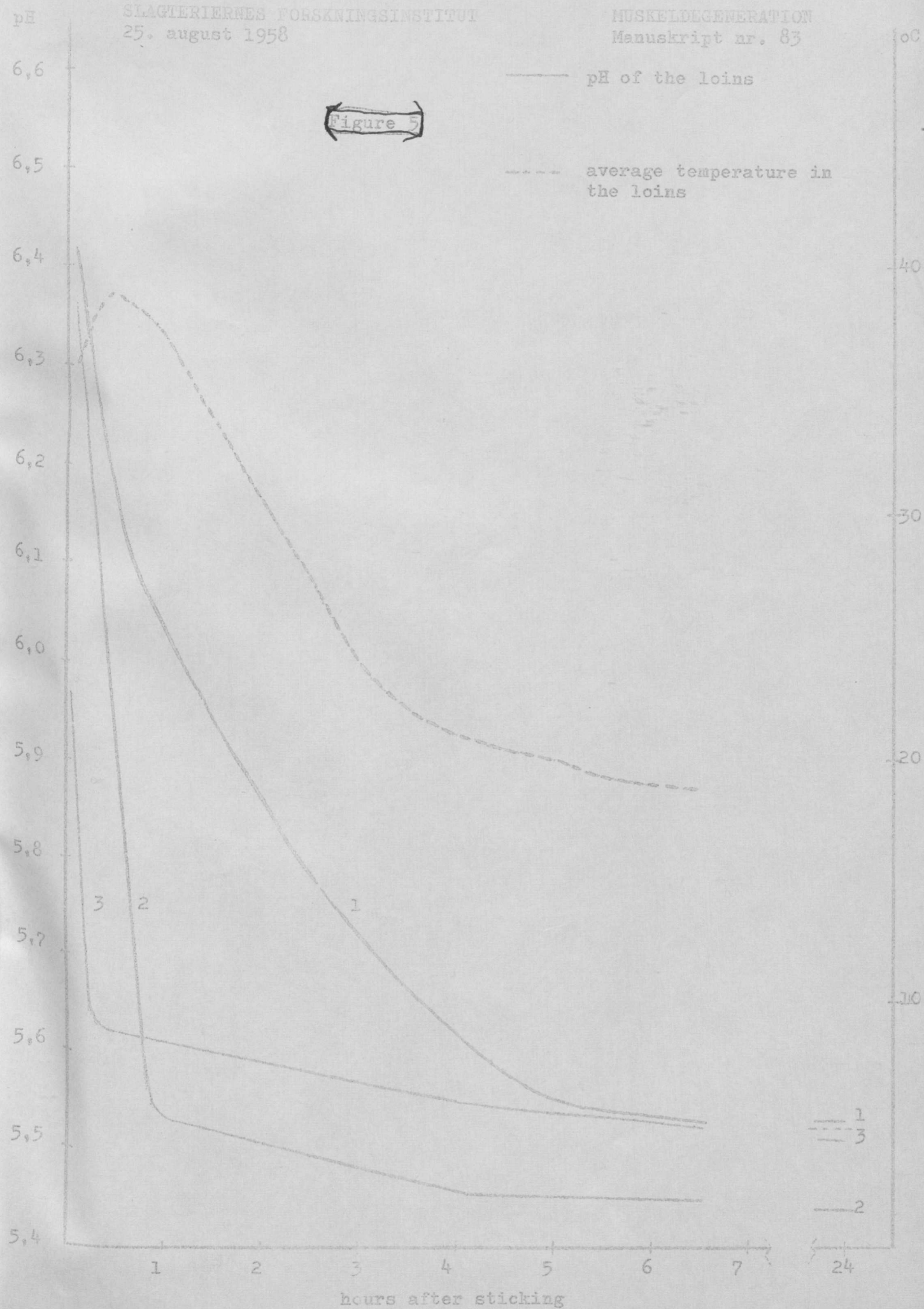
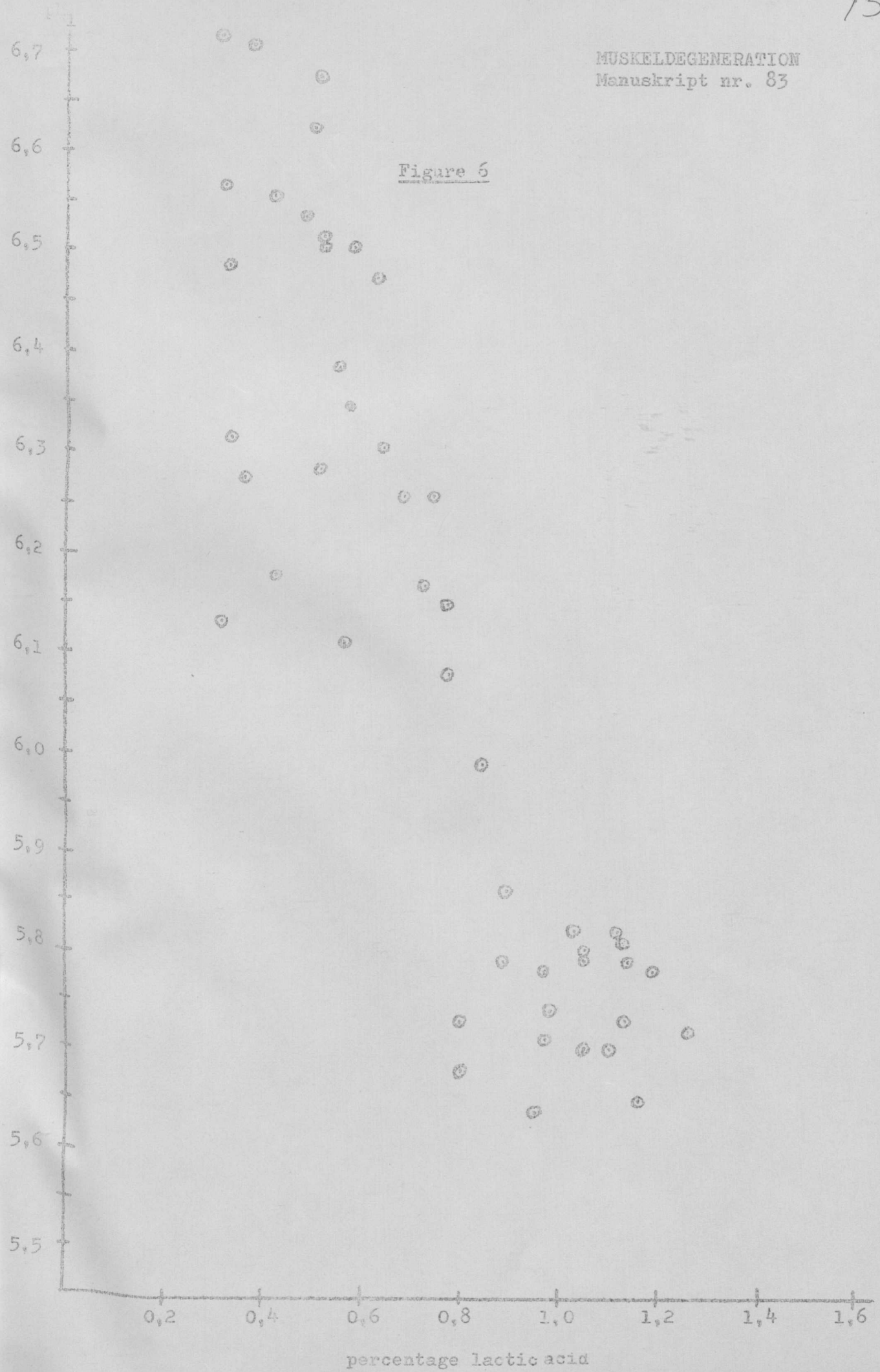


Figure 6



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Manuskript nr. 83

Figure 7

percentage of
added glycogen
decomposed during
incubation

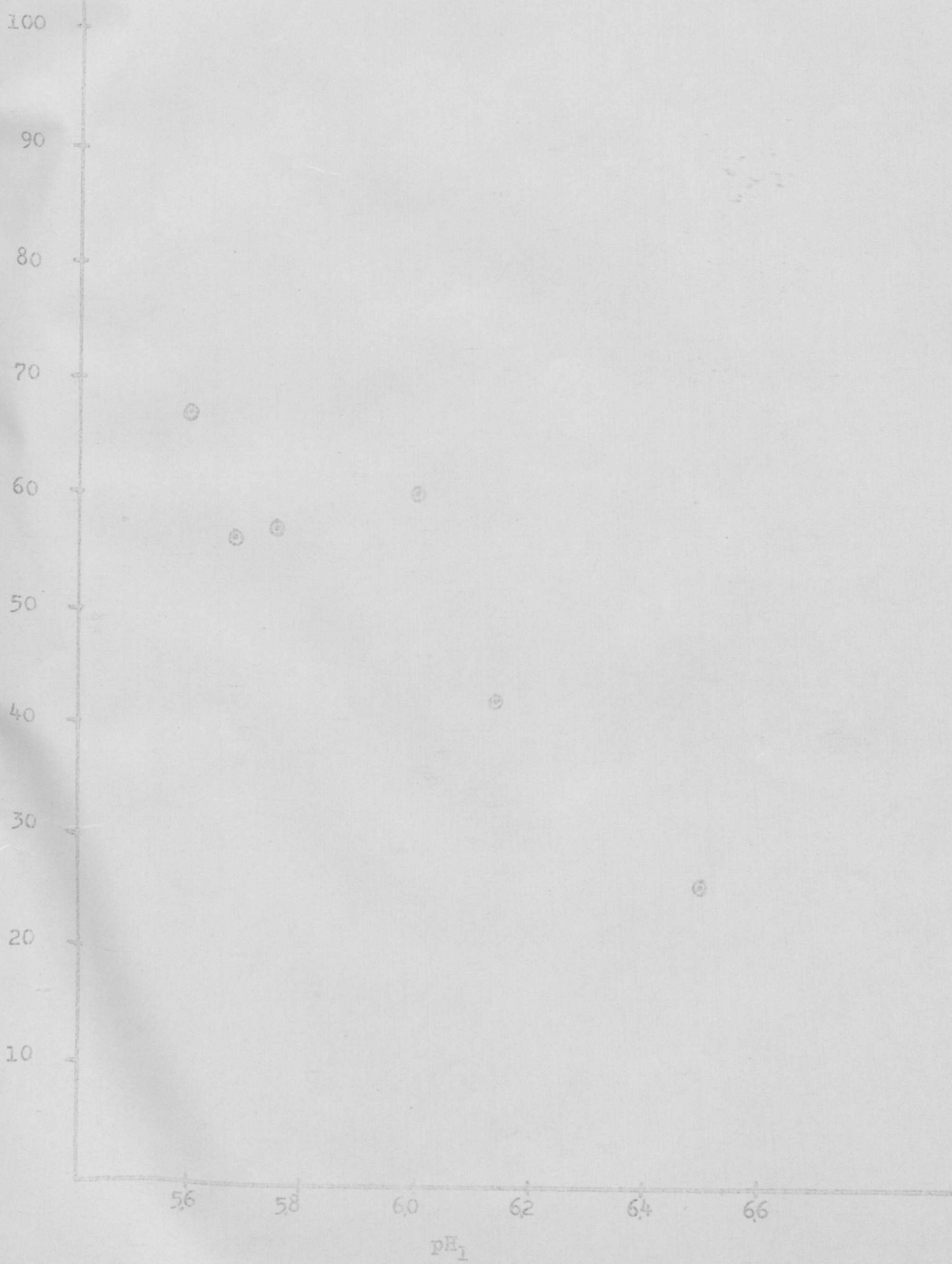
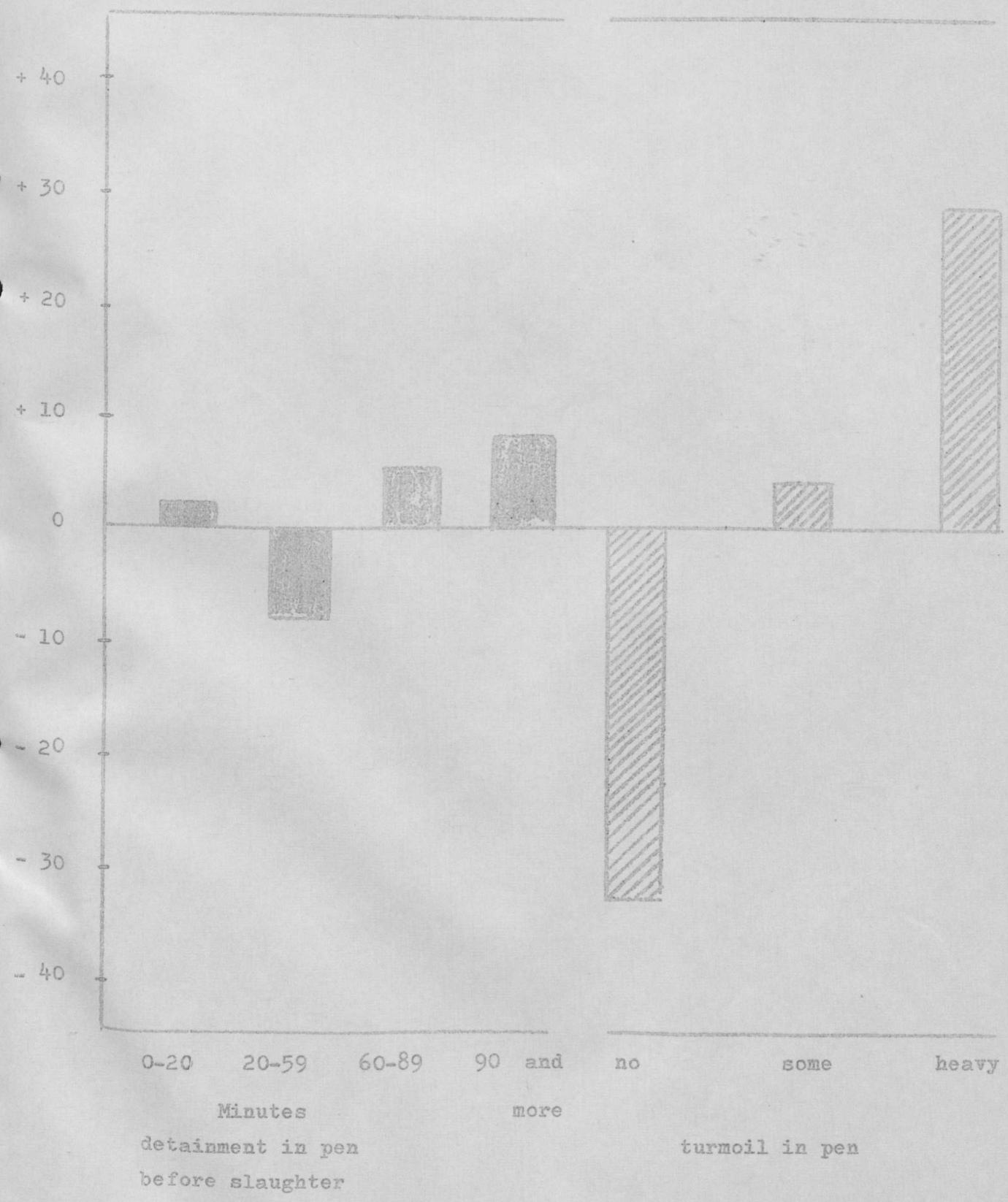


Figure 8

percentage increase or
decrease of loins with $pH_1 < 6,2$



percentage increase or
decrease of loins with $pH_1 < 6.2$



Figure 9

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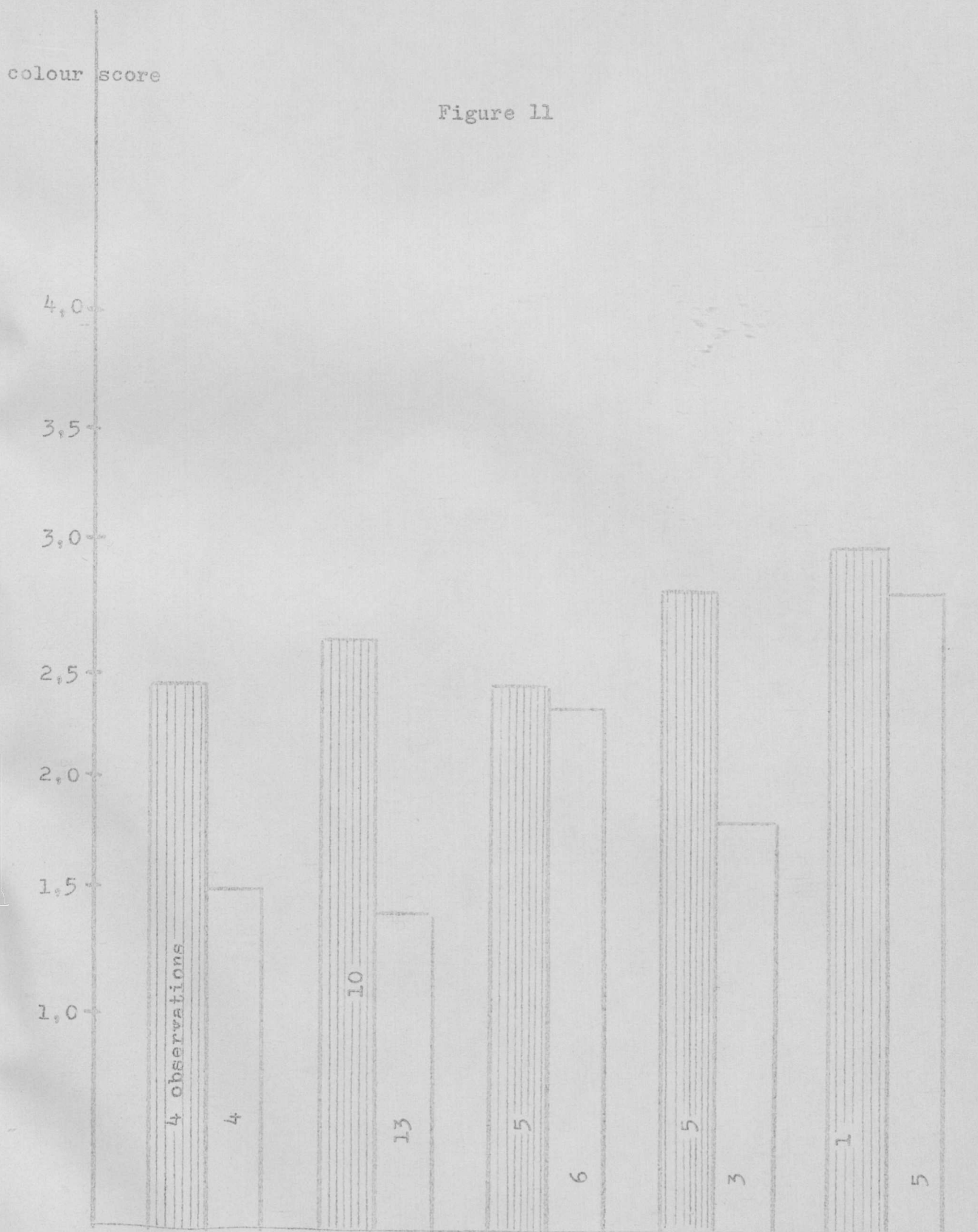
MUSKELDEGENERATION
Manuskript nr.83



Figure 10

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MUSKELDEGENERATION
Manuskript nr. 83

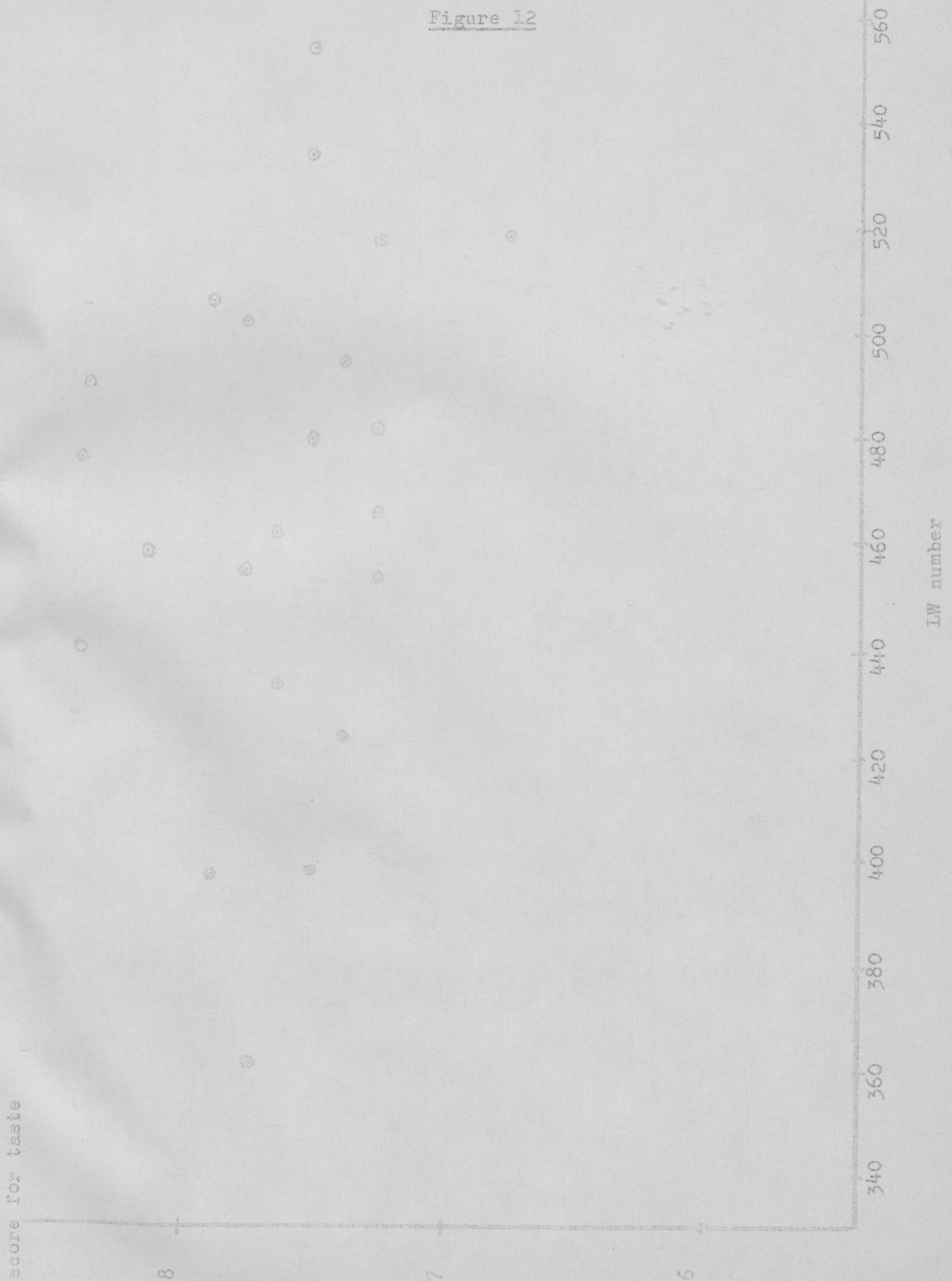


LW number	360- 460- 461- 560-	360- 460- 461- 560-	360- 460- 461- 560	360- 460- 461- 560-	360- 460- 461- 560-
haematin	0,080-	0,100-	0,120	0,140	0,160
mg/g meat	0,099	0,119	0,139	0,159	0,179

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25. august 1958

MUSKELDEGENERATION
Manuskript nr. 83

Figure 12

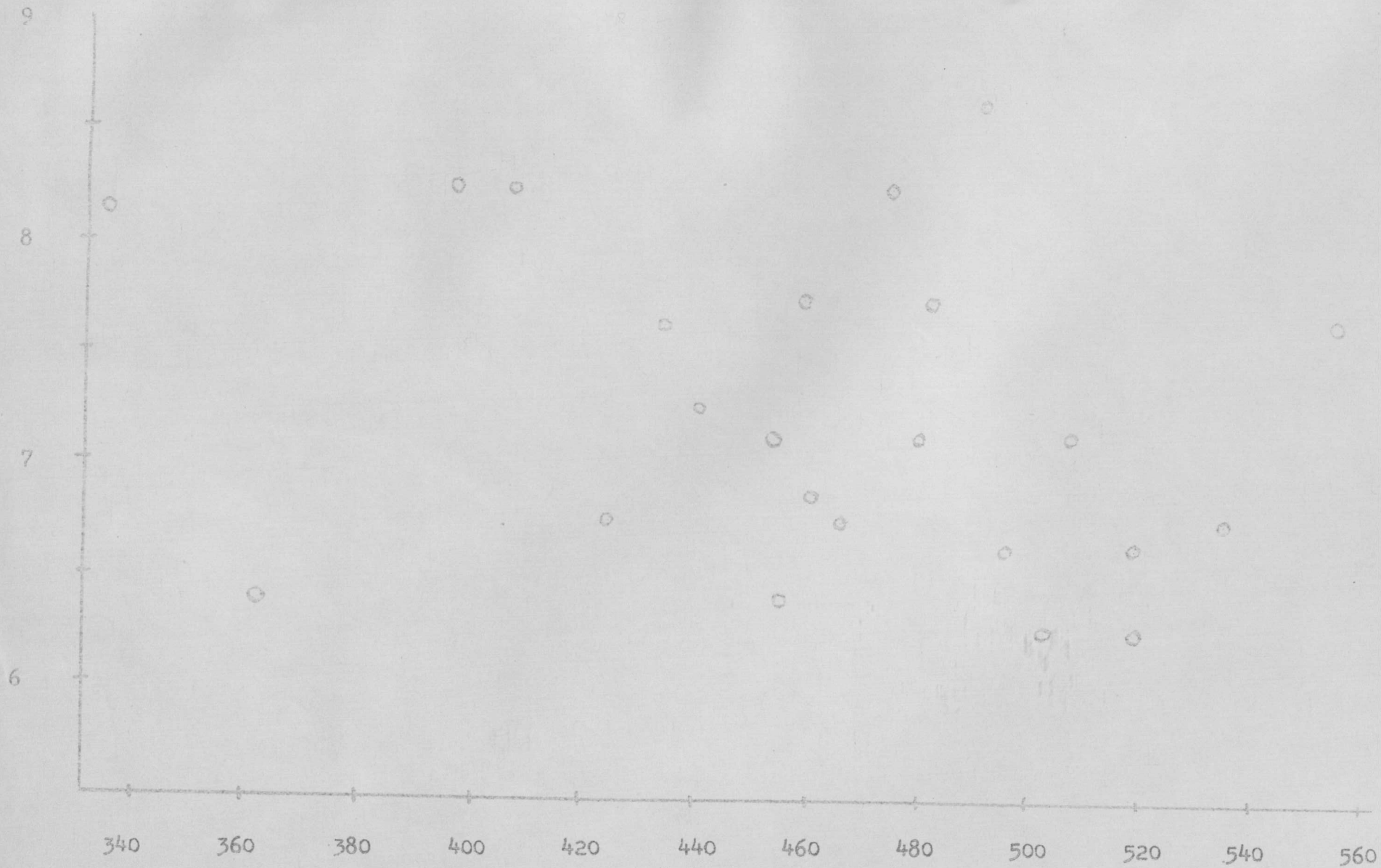


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25. august 1958

MUSKELDEGENERATION
Manuskript nr. 83

137

score for texture



LW number
Figure 13