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IDENTIFICATION OF IRRADIATED MEAT BY THIN-LAYER GEL CHROMATOGRAPHY AND SOLUBILITY STUDIES

by

B.J. RADOLA
(Institut für Strahlentechnologie, Karlsruhe)

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ABSTRACT

In beef and pork irradiated with a dose of 1 and 5 Mrad a new radiation-induced, sarcoplasmic protein fraction was observed on thin-layer gel chromatography on Sephadex G-200. The radiation-induced fraction migrating ahead of all other sarcoplasmic protein fractions of untreated meat could be readily identified in the chromatographic pattern. The amount of the radiation-induced fraction increased with increasing dose. Following irradiation at — 30 °C a smaller amount of this fraction was found than at 0 °C. Solubility studies based on stepwise extraction of a protein precipitate absorbed on an inert carrier with a series of ammonium sulphate solutions of decreasing molarity have shown that the fractions extracted with 1, 1.5 and 2 M ammonium sulphate are reduced in irradiated meat. The demonstration of a radiation-induced, sarcoplasmic protein fraction can serve as a basis for identification of meat irradiated with a dose of 5 Mrad.

KEYWORDS

THIN-LAYER CHROMATOGRAPHY
PORK
GAMMA RADIATION
IRRADIATION
PRESERVATION
DOSE RATES
RADIATION EFFECTS
SOLUBILITY
MYOSIN
MYOGLOBIN
LOW TEMPERATURE
PROTEINS

FOREWORD

In order to be able to check on the observance of public health regulations, *practical and effective* testing methods must be available for inspecting foodstuffs irradiated for preservation purposes. Generally speaking, these techniques are not yet fully developed as has been demonstrated in a study carried out at our request by A. Lafontaine and L. Bugyaki : "Etude sur les méthodes d'identification des denrées alimentaires irradiées" (Report EUR 2402 f/1965).

At the beginning of 1967, the Commission's Health Protection Directorate concluded a number of research contracts with competent laboratories and research institutes in the European Community with the aim of studying physico-chemical and biological changes in irradiated foodstuffs. The main purpose of this research is to develop analytical methods for identifying irradiated foodstuffs.

Dr. Radola's research proves that it is possible to demonstrate measurable changes, stable in time, in certain constituents of irradiated meat. Because of the significance and importance of this work, we felt that a publication would be of great interest, as it was the case for Dr. Deschreider's report : "Modifications des constituants de la farine irradiée mises en évidence par spectrophotométrie, spectropolarimétrie et analyse thermodifférentielle" (Report EUR 4417 f/1970). Dr. Radola is to be congratulated on the quality of his research and for the excellent results he has achieved.

Further reports will be appearing on other irradiated foodstuffs and on other analytical methods which are being studied within the framework of these contracts.

Dr. P. Recht

CONTENTS

IDENTIFICATION OF IRRADIATED MEAT BY THIN-LAYER GEL CHROMATOGRAPHY AND SOLUBILITY STUDIES (*)

1 — INTRODUCTION

Radiation-induced aggregation has been observed for a number of homogeneous proteins (1 - 8) and for model systems composed of two proteins differing in physicochemical properties (9). The dose dependence and specificity of radiation-induced aggregation and the stability of the aggregates appeared promising as a base for identification of irradiated protein-rich food.

By use of a variety of methods radiation-induced aggregates can be detected (2, 4, 5, 6, 8). Gel chromatography, a method separating substances according to differences in molecular size, affords the advantage of high resolution and relative simplicity. A thin-layer technique of gel chromatography has been described (10 - 13) and it appeared to be of potential utility for routine analysis. Meat has been chosen as a model food for our studies because of its technological importance and its high protein content which was considered to be a methodological advantage. A number of factors which may be anticipated to affect the radiation response were studied. These included : the quality of radiation, the dose rate and the irradiation temperature. Our results indicate that all these factors have an influence on the effects observed and are therefore of potential importance in any consideration of identification methods of irradiated food.

Evidence for changes in solubility of proteins in irradiated food has been obtained, rendering extraction of the soluble proteins a critical step for further analysis. An attempt was made, therefore, to study in a more direct way the effect of irradiation on the solubility of proteins. A method based on stepwise extraction of the proteins precipitated by saturated ammonium sulphate solutions by a series of ammonium sulphate solutions of decreasing molarity was used for this purpose (14, 15).

2 — MATERIALS AND METHODS

Fresh beef and pork (longissimus dorsi) was irradiated with a dose of 1 and 5 Mrad either in closed glass vessels or in sealed polyethylene bags. Two irradiation sources were used : 1) a cobalt source with a dose rate of $1.0\text{-}1.5.10^4$ rad/min, and 2) an electron accelerator (Varian); the energy of the electrons was 10 MeV and the mean pulse dose rate $\sim 10^{11}$ rad/sec. The irradiation temperature was 0 or — 30 °C. The irradiated meat was analyzed within a few days after irradiation or after storage for several weeks at room temperature.

For preparation of sarcoplasmic proteins 10 g of meat were homogenized for 2-3 min with 30 ml of ice-chilled 0.1 M phosphate buffer (KH_2PO_4-Na_2HPO_4)pH 7.2-7.4 in a Waring blendor with cooling. The homogenates were centrifuged at 4 °C at 35000 g for 15 min. The super-

(*) Manuscript received on December 1, 1970.

natant was either concentrated directly or dialyzed first against 1 % glycin followed by ultrafiltration on UM-10 or PM-10 Diaflo membranes (Amicon, N.V. Oosterhout, Holland) to an optical density of $E\,^{1\,cm}_{280} = 50$, corresponding to a protein concentration of 4-5 %. The concentrated solutions were analyzed within a few days. If kept longer they were preserved with merthiolate 1 : 5000 or 1 : 10000 and kept a 4 °C.

Thin-layer gel chromatography was performed essentially as described elsewhere (12, 13). Briefly, 20 × 20 cm glass plates were coated with a 0.5 mm layer of a suspension of Sephadex G-75, G-100 or G-200, all "superfine" (Pharmacia, Uppsala, Sweden), in a 0.02 M phosphate buffer pH 7.2-7.4 containing 0.5 M NaCl. After equilibration overnight 10-20 µl of the concentrated solutions of sarcoplasmic protein were applied. Sperm whale myoglobin and ferritin were run as reference proteins. The flow rate was adjusted to 1 cm/hr for myoglobin. After 4-6 hours the separation was stopped and a print was taken with chromatographic paper Whatman No. 3 or occasionally Whatman No. 1 with the G-75 gel. After contact with the gel for 20-30 sec, the print was removed, dried at 110 °C for 15 min and stained in a 9 : 1 v/v methanol-acetic acid solution of amido black 10 B (E. Merck, Darmstadt) or Coomassie Blue R 250 (Serva, Entwicklungslabor, Heidelberg). The prints were destained with a mixture of methanol-acetic acid-water 50 : 10 : 40 v/v. Densitometry (in reflectance) was accomplished either with the Chromoscan densitometer equipped with the thin-layer attachment (Joyce-Loeble & Comp. Ltd., Gateshead, England) or with the Zeiss Chromatogram Spectrophotometer (C. Zeiss, Oberkochen, Germany).

For molecular weight determinations the distance from the starting line to the middle of each zone was measured with an accuracy of 0.5 mm either directly on the print or on the densitogram. The results were expressed by the R_M value defined as the ratio of the migration distance of the sarcoplasmic protein fraction (d_P) to that of myoglobin (d_M) which was used as the reference protein

$$R_M = \frac{d_P}{d_M}.$$

Solubility experiments : 3 g of meat (untreated or irradiated) were homogenized for 3 min at 0 °C in a MSE homogenizer with 40 ml of a 4 M ammonium sulphate solution, pH 7.0-7.5. Six grams of cellulose powder (MN Cellulose Pulver, Macherey-Nagel & Co., Düren, Germany) were added as an inert carrier. Twenty ml of the cellulose suspension containing the protein precipitate were placed into a 20 × 1 cm column and allowed to settle. Proteins were extracted from the column with a series of ammonium sulphate solutions of decreasing molarity, 50 ml of each solution being used. Fractions of ~ 5 ml/10 min were collected by means of a fraction collector at a constant flow established by using a peristaltic pump. Absorbancy readings were performed at 280 nm. For some of the fractions absorbancies at two wave lengths were measured.

3 — RESULTS

In preliminary experiments optimal conditions for the separation of sarcoplasmic proteins by thin-layer gel chromatography were determined. With the G-75 gel only two zones were detected — one corresponded to myoglobin (R_M value = 1.0), while the other had an R_M very close to the exclusion limit of this particular gel (Fig. 1). There was no difference in the pattern between the untreated material and the sample irradiated with 5 Mrad. With the G-100 gel besides myoglobin three other components with higher R_M values were observed (Fig. 2). Densitometrically only two peaks could be obtained, one of myoglobin and the other, a strongly asymmetric one, corresponding to the components with higher molecular weights. Meat irradiated with 5 Mrad showed consistently an increased content of fraction F3 which formed the leading part of the asymmetric peak (Fig. 2B).

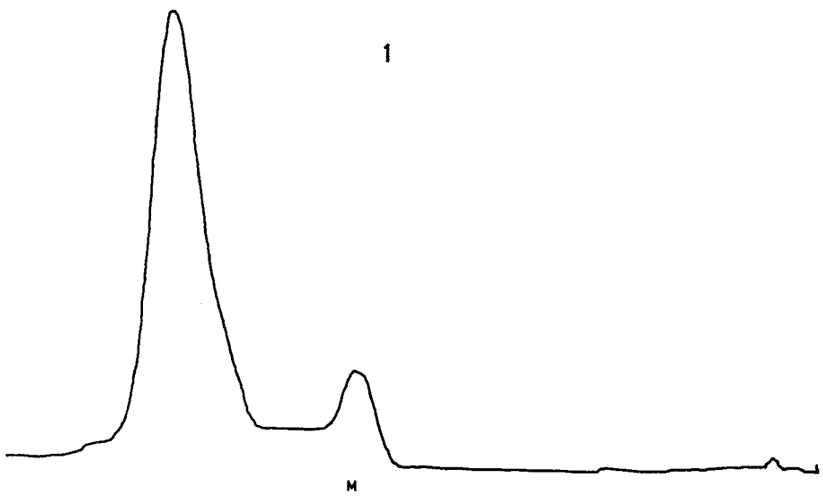
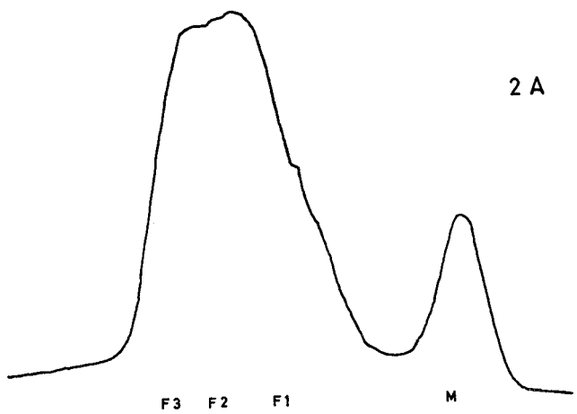


Fig. 1 — Thin-layer gel chromatography of beef sarcoplasmic proteins on Sephadex G-75. M-myoglobin.

A — untreated meat



B — meat irradiated with 5 Mrad

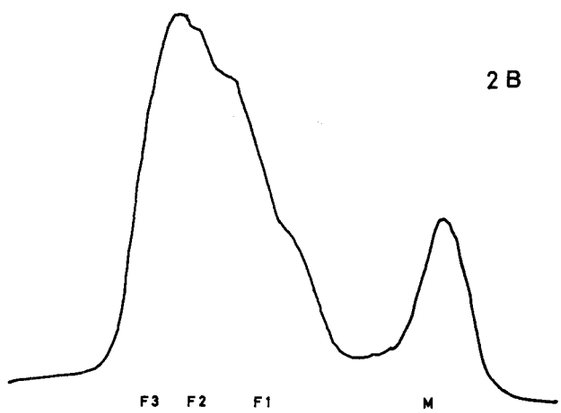
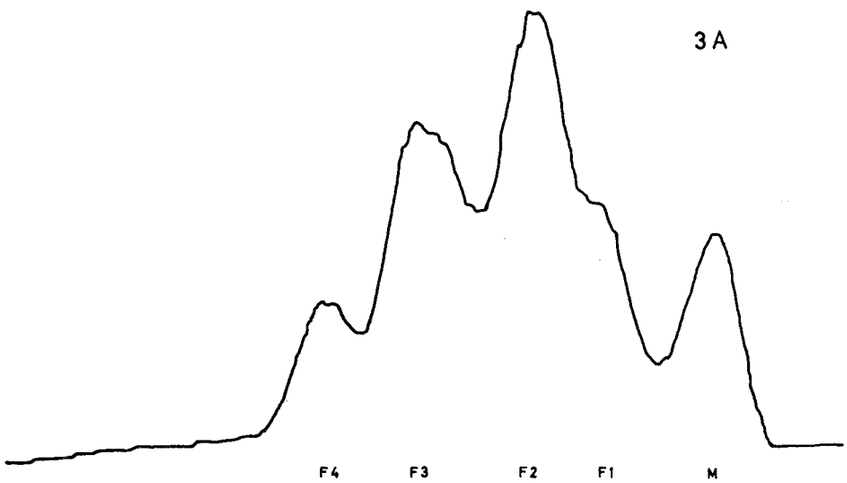


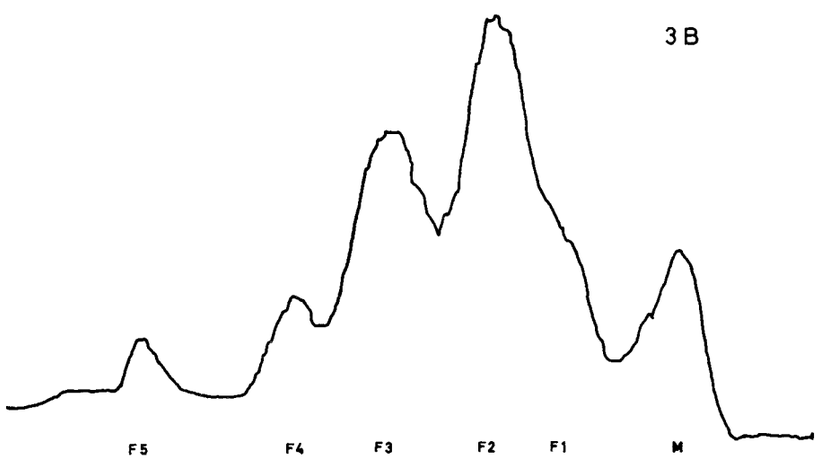
Fig. 2 — Thin-layer gel chromatography of beef sarcoplasmic proteins on Sephadex G-100. M-myoglobin, F1 - F3-fractions with increasing R_M value :

The best resolution was achieved with the G-200 gel. Sarcoplasmic proteins of beef and pork could be resolved into five fractions termed F1 - F4 according to increasing R_M value (Fig. 3). In irradiated meat an additional fraction F5 appeared which migrated ahead of the most rapid fraction F4 of the untreated meat (Fig. 3B and 3C). This radiation-induced fraction with an R_M value of $\sim$ 2.4 could be detected both in samples irradiated with 1 and 5 Mrad the percentage content of the fraction increasing with the dose. In Tables I and II the R_M values for the sarcoplasmic protein fractions of beef and pork are presented for the untreated meat and for meat irradiated

A — untreated meat



B — meat irradiated at 0 °C with 1 Mrad



C — meat irradiated at 0 °C with 5 Mrad

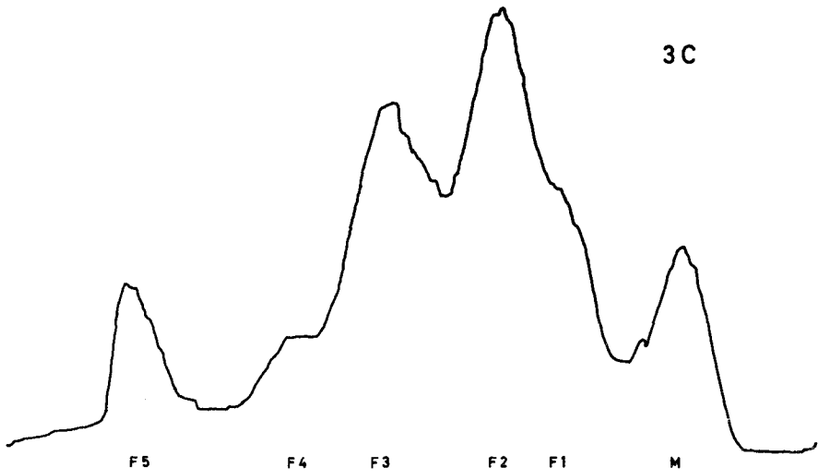


Fig. 3 — Thin-layer gel chromatography of beef sarcoplasmic proteins on Sephadex G-200. M-myoglobin, F1 - F5-fractions with increasing R_M values :

with 1 and 5 Mrad. The molecular weights have been calculated for the fractions of unirradiated meat according to the following equation (12) :

$$\log M = 1.172\ R_M + 3.015.$$

Only minor differences were noted in the R_M values of the fractions in irradiated meat indicating that there are no changes in the size properties of fractions F1 - F4. The R_M value of the radiation-induced fraction corresponds to the exclusion limit of the G-200 gel. Due to this high R_M value the radiation-induced fraction could be easily identified in the pattern of sarcoplasmic proteins. Beef and pork sarcoplasmic proteins showed very similar patterns with only quantitative variations in the content of some fractions. The results presented in Tables I and II demonstrate that there are also only small differences in the R_M values.

TABLE I

R_M-Values and molecular weights of beef sarcoplasmic proteins determined by thin-layer gel chromatography on Sephadex G-200

			Irradiation at 0 °C		Irradiation at — 30 °C	
Fraction	Unirradiated	Molecular weight (1)	1 Mrad	5 Mrad	1 Mrad	5 Mrad
F 1	1.36	41000	1.36	1.37	1.36	1.39
F 2	1.55	68000	1.53	1.55	1.53	1.55
F 3	1.87	157000	1.86	1.87	1.86	1.84
F 4	2.15	340000	2.09	2.12	2.09	2.09
F 5	—		2.45	2.40	2.45	2.30

(1) Calculated for the R_M values of unirradiated meat. The R_M values were obtained from 6-12 determinations.

TABLE II

R_M-Values and molecular weights of pork sarcoplasmic proteins determined by thin-layer gel chromatography on G-200

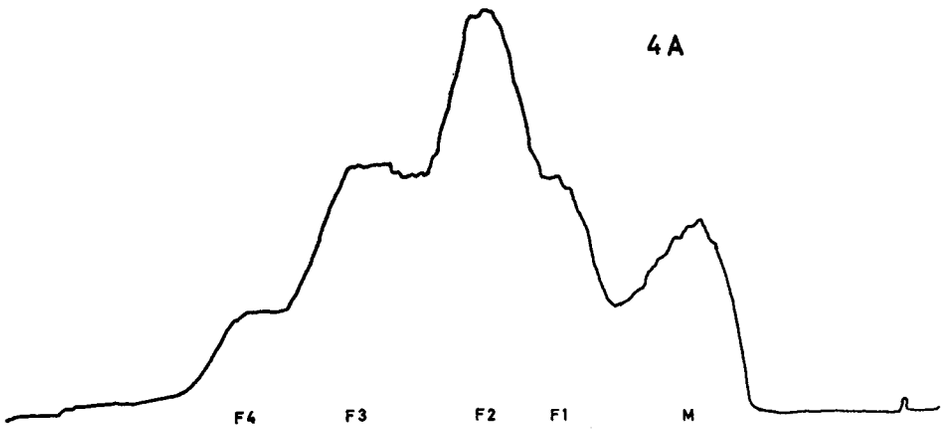
Fraction	Unirradiated	Molecular weight (1)	Irradiation at 0 °C		Irradiation at — 30 °C	
			1 Mrad	5 Mrad	1 Mrad	5 Mrad
F 1	1.34	39000	1.35	1.36	1.36	1.39
F 2	1.52	63000	1.53	1.54	1.55	1.57
F 3	1.82	143000	1.81	1.84	1.85	1.87
F 4	2.09	300000	2.07	2.10	2.09	2.11
F 5	—		2.45	2.39	—	2.41

(1) Calculated for the R_M values of unirradiated meat. The R_M values were obtained from 6-12 determinations.

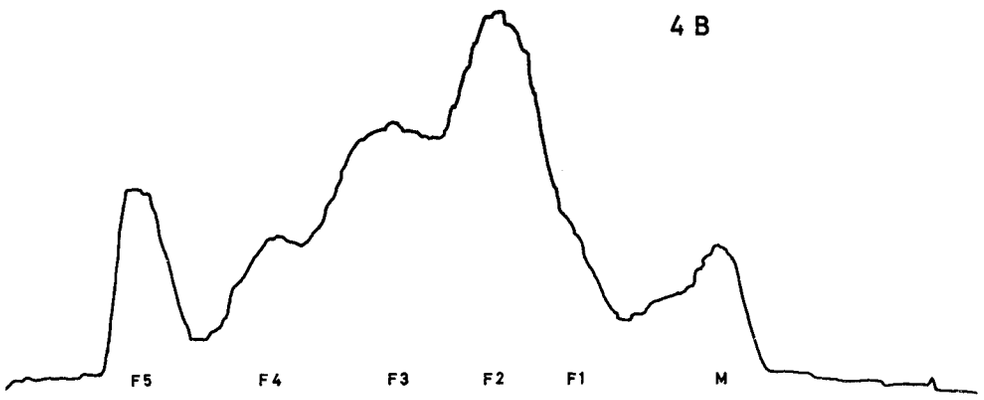
In storage experiments meat irradiated with a dose of 5 Mrad was kept at room temperature for periods up to 10 weeks. On thin-layer gel chromatography sarcoplasmic proteins isolated from the stored meat still contained the radiation-induced fraction thus proving the stability of this fraction. In these experiments meat samples preserved at — 30 °C for comparable periods were used as a control. No fraction with the $R_M \sim 2.4$ value was observed in the frozen meat after prolonged storage. In additional experiments meat was subjected to repeated freezing (— 30 °C) and thawing cycles, a treatment considered to be more effective for the induction of aggregates than simple storage for prolonged periods at low temperatures. Following this treating only traces if any of the $R_M \sim 2.4$ fraction could be detected in the sarcoplasmic extract. These results indicate that aggregation of sarcoplasmic proteins appears to be specific for irradiated meat, an important result for distinguishing irradiated meat from that preserved in frozen state.

Distinct differences in the content of this radiation-induced fraction were observed in the 1 and 5 Mrad samples. These differences could provide a basis for dosimetry. However, results obtained on irradiation at low temperature as well as dose rate experiments indicate that this dosimetry should be interpreted with caution. It is well established that irradiation of protein-rich and even of some protein-poor food products at room temperature will induce undesirable organoleptic changes. These changes may be suppressed by irradiation at a sufficiently low temperature. Thus meat irradiated at — 70 to — 30 °C yielded an organoleptically acceptable product. Irradiation at — 30 °C is now considered as adequate. When beef and pork were irradiated at — 30 °C and subsequently the soluble proteins analyzed by thin-layer gel chromatography on Sephadex G-200 a fraction with $R_M \sim 2.4$ was observed in both samples. The amount of this fraction was consistently found to be reduced when irradiation was carried out at — 30 °C (Fig. 4). These results probably reflect differences in the physico-chemical state of the proteins in meat, some of the soluble proteins being not available for radiation-induced aggregation in the frozen state. The temperature of irradiation thus appears to be an important factor in identification : at lower doses (1 Mrad) and at lower temperatures (— 30 °C) the radiation induced aggregation was strongly reduced and the irradiated samples could not be easily recognized from the untreated sample.

A — untreated meat



B — meat irradiated with 5 Mrad at 0 °C



C — meat irradiated with 5 Mrad at —30 °C

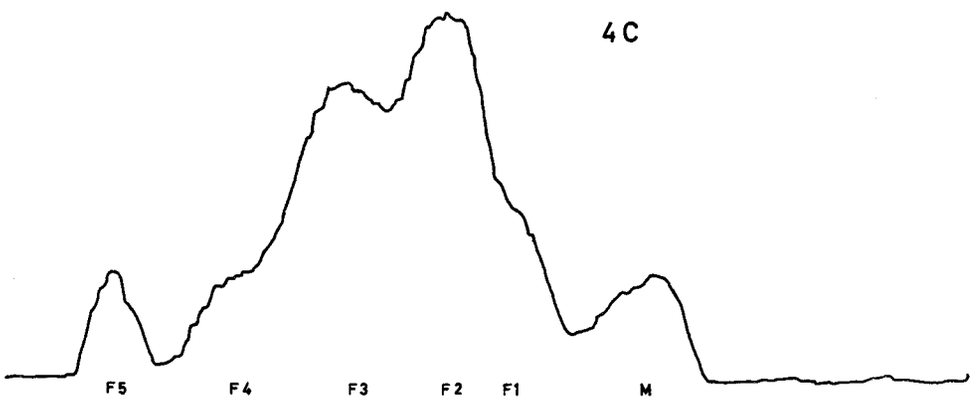


Fig. 4 — Effect of irradiation temperature.

Thin-layer gel chromatography of pork sarcoplasmic proteins on Sephadex G-200. M-myoglobin, F1 - F4-fractions with increasing R_M values :

Dose rate was found to be another important factor. Irradiation of beef and pork at two mean pulse dose rates 10^{10} and 10^{11} rad/sec both yielded the $R_M \sim 2.4$ fraction. The amount of this fraction was reduced at the lower dose rate. This is presented in Fig. 5. Dose rates of this order are rather high and exceed those of conventional cobalt sources by a factor of 10^4-10^5. These results demonstrate that by the dose alone the irradiated material is not sufficiently characterized. Additional work is needed to compare the results obtained on irradiation in conventional cobalt sources with those of other irradiation sources.

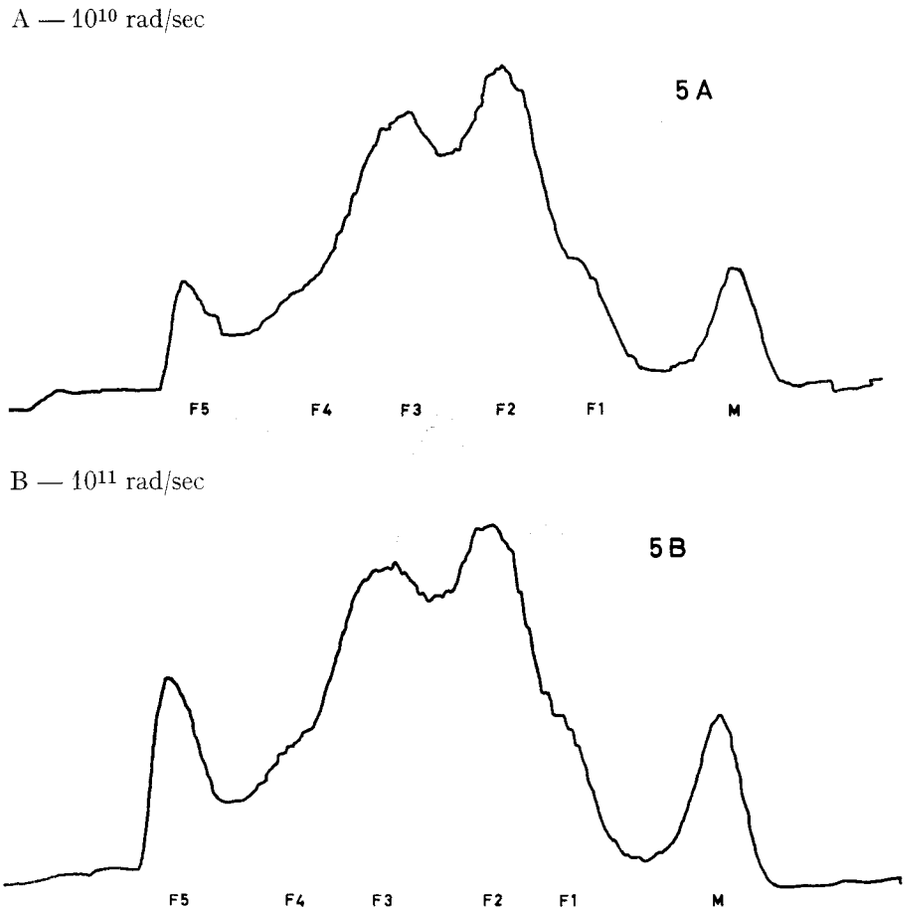
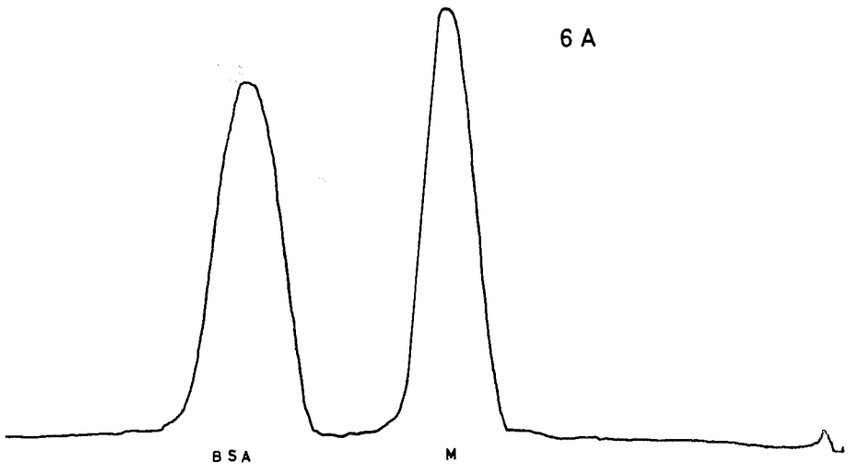


Fig. 5 — Effect of dose rate

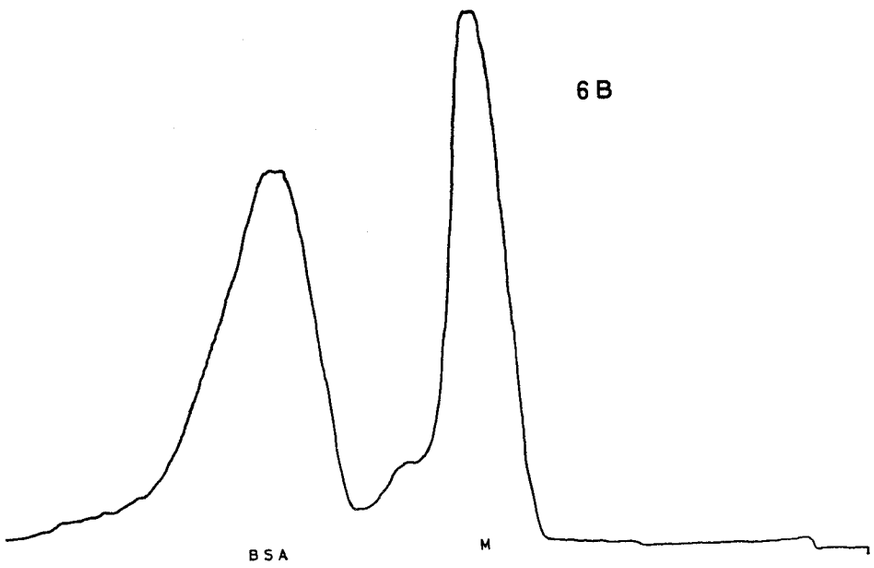
Thin-layer gel chromatography of beef sarcoplasmic proteins on Sephadex G-200. Irradiation at 0 °C with 5 Mrad in a linear accelerator with a mean pulse dose rate:

The experiments described above have consistently shown that on thin-layer gel chromatography of sarcoplasmic proteins from irradiated meat a fraction with an increased R_M value appears, which has been tentatively described as radiation-induced fraction. A number of experiments were performed in order to prove the hypothesis that this fraction results from aggregation of sarcoplasmic proteins. In Fig. 6 thin-layer gel chromatography patterns are presented for a mixture of 1 % myoglobin and 1 % serum albumin irradiated in phosphate buffer with increasing doses in a cobalt source. The results indicate that with increasing dose the amount of aggregates increases. For the lowest dose (0.5 Mrad) a small amount of dimerized myoglobin and a strong asymmetry of the albumin peak were observed (Fig. 6B). For the highest dose (2 Mrad) almost all the proteins appear as a fraction with an R_M value of ~ 2.4 with only small amounts of residual myoglobin and traces of albumin (Fig. 6D).

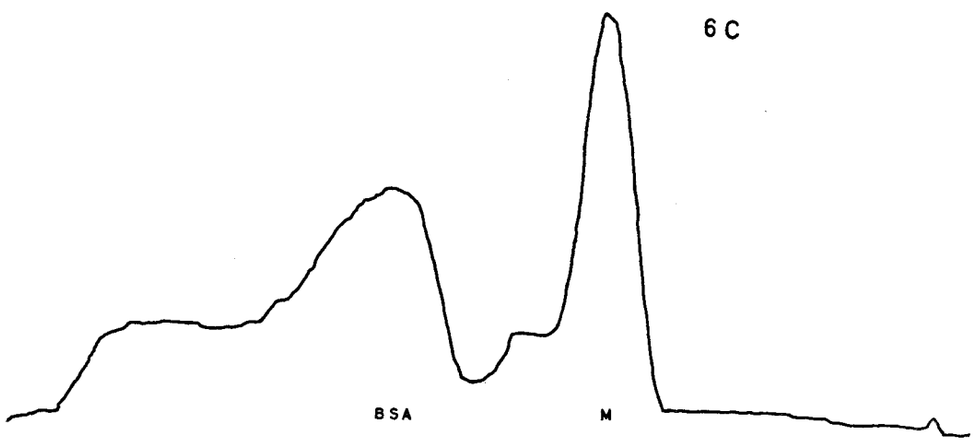
A — unirradiated



B — irradiated with 0.5 Mrad



C — irradiated with 1 Mrad



D — irradiated with 2 Mrad

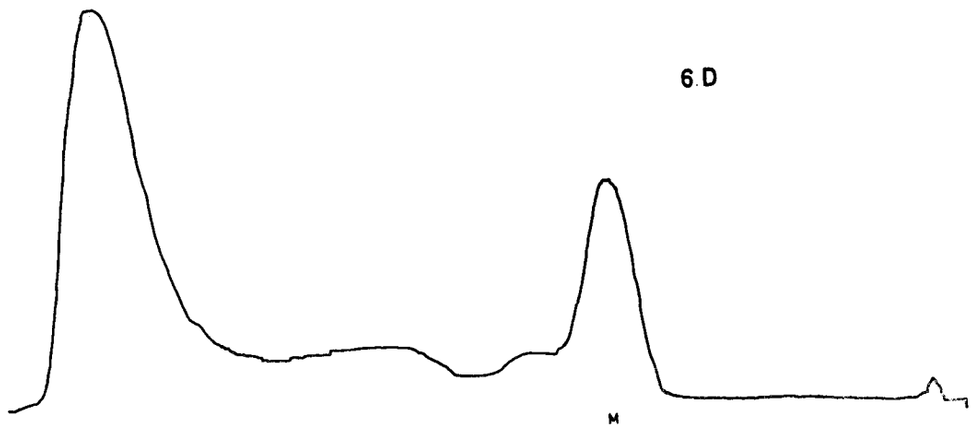
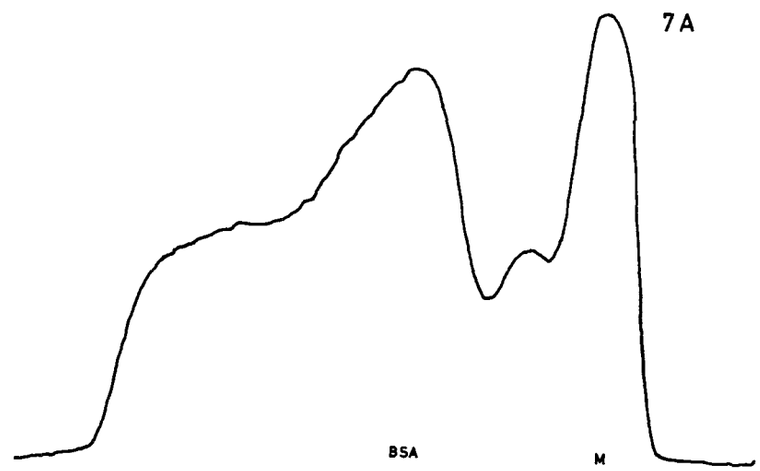


Fig. 6 — Radiation-induced aggregation in a model system of two proteins.
Thin-layer gel chromatography on Sephadex G-200 of a mixture of 1 % bovine serum albumin (BSA) and sperm whale myoglobin (M) irradiated (cobalt source) in 0.02 M phosphate buffer.

The radiation induced aggregation depended on the concentration of the protein mixture. In Fig. 7 patterns are shown of myoglobin and albumin mixtures containing 2, 1, and 0.5 % of each protein irradiated with a constant dose of 1 Mrad. The patterns clearly demonstrate that while at the highest protein concentration only a small fraction of the proteins is aggregated (Fig. 7A), with the lowest protein concentration almost all the protein has aggregated (Fig. 7C). The aggregated material migrated with an R_M value of $\sim$ 2.4. These findings are important for the interpretation of results obtained on irradiation of food products in which often relatively high protein concentrations will be present.

Total protein concentration :

A — 2 %



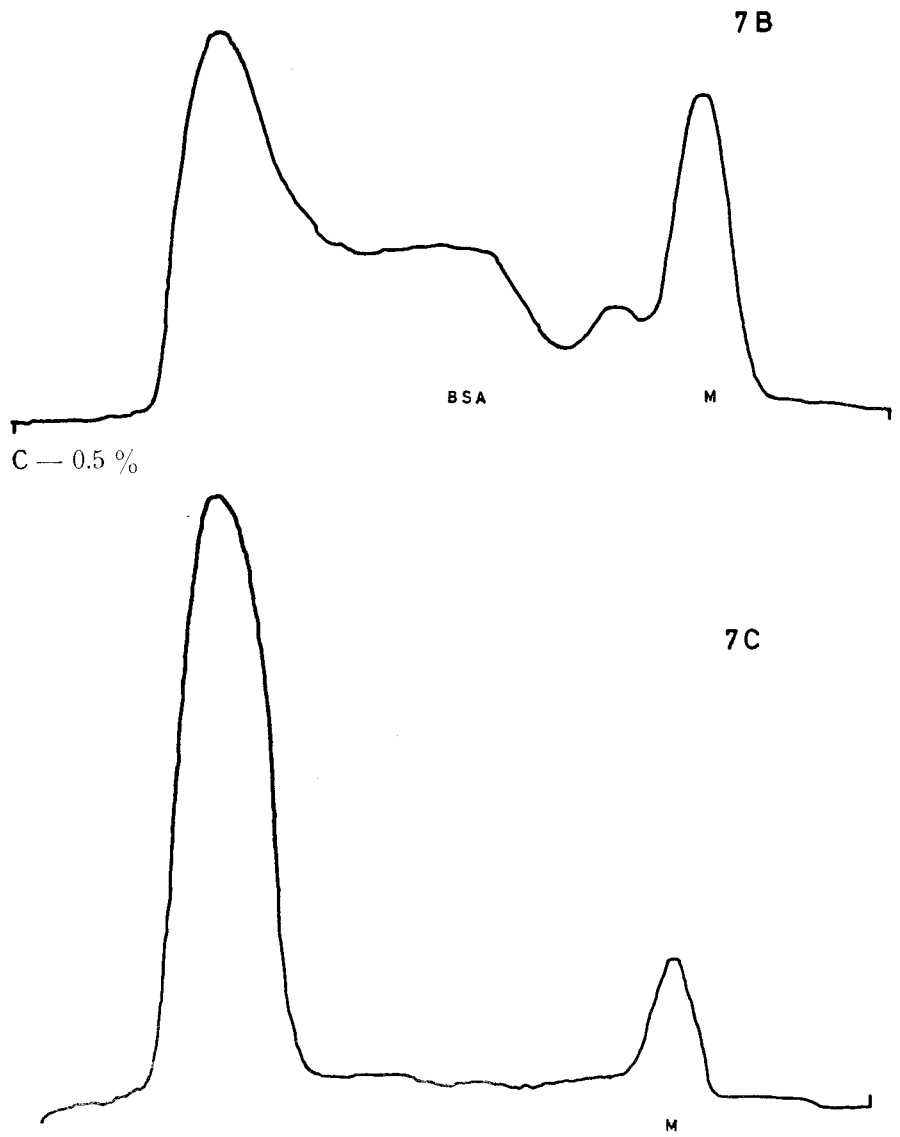
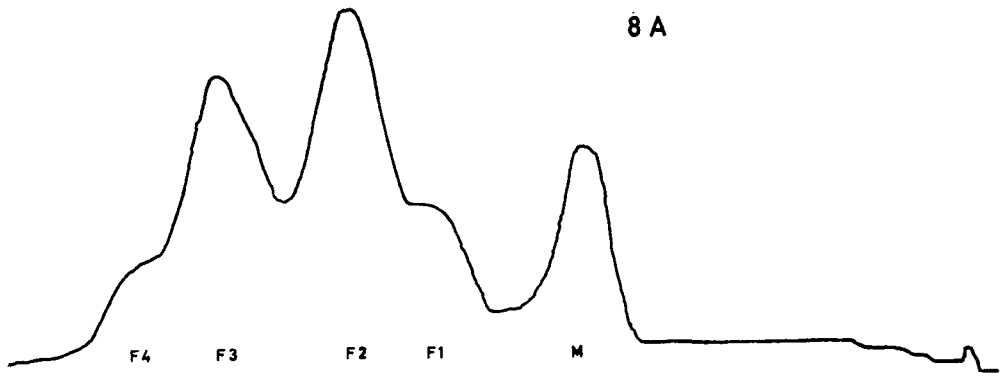


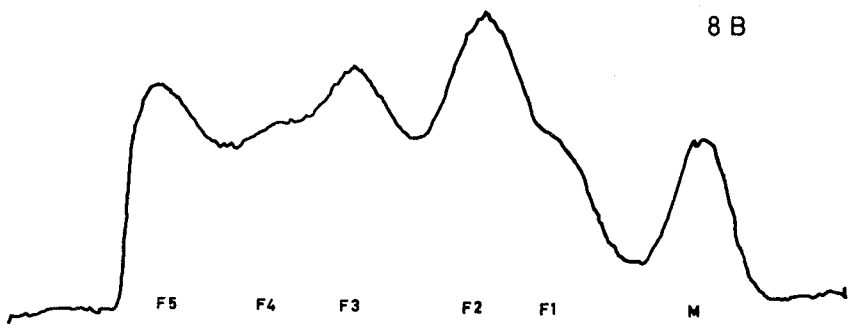
Fig. 7 — Effect of protein concentration on radiation-induced aggregation in a model system of two proteins.
Thin-layer gel chromatography on Sephadex G-200 of mixture of bovine serum albumin (BSA) and sperm whale myoglobin (M) irradiated with 1 Mrad in 0.02 M phosphate buffer.

In addition to these experiments with a model system of two proteins, further experiments were performed with isolated beef sarcoplasmic proteins. The sarcoplasmic proteins were obtained by extraction with 0.1 M phosphate buffer and dialyzed 48 hours against a 0.01 M phosphate buffer of the same pH. The dialyzed solution was concentrated by ultrafiltration on UM-10 Diaflo membranes to an $E\,^{1\,cm}_{280} = 50$ value. The protein solution was irradiated in sealed ampoules at 0 °C in a cobalt source (Fig. 8). Already at the lowest dose, namely 0.5 Mrad, a new fraction appears with an R_M value identical with that of the radiation induced fraction in irradiated meat (Fig.8B). At the highest dose — 2 Mrad the radiation-induced fraction was the dominating fraction of the pattern and only small residual amounts of fractions F2 and M were observed (Fig. 8D). These results obtained on irradiation of sarcoplasmic proteins provide strong support for the assumption that the radiation-induced fraction observed in extracts of irradiated meat results from aggregation of sarcoplasmic proteins.

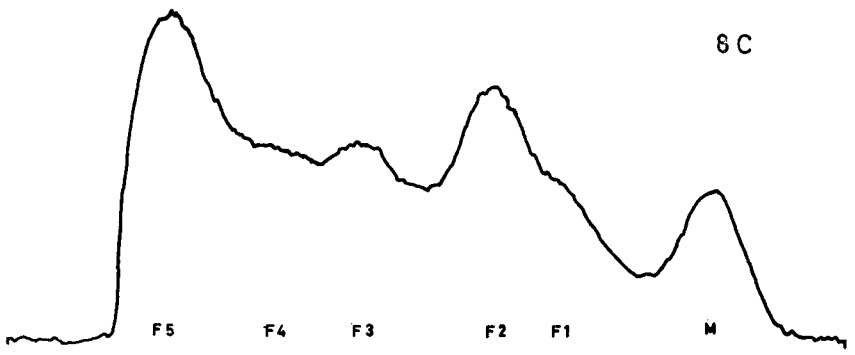
A — unirradiated sarcoplasmic proteins



B — irradiated with 0.5 Mrad



C — irradiated with 1 Mrad



D — irradiated with 2 Mrad

Fig. 8 — Radiation-induced aggregation of beef sarcoplasmic proteins irradiated in 0.02 M phosphate buffer pH 7.2.

Thin-layer gel chromatography on Sephadex G-200.

M-myoglobin, F1 - F4-fractions with increasing R_M value, F5 - radiation-induced fraction.

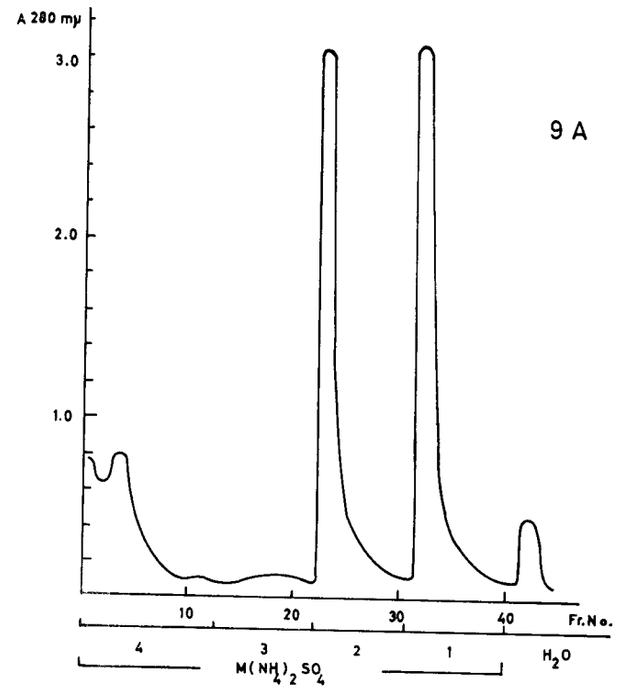
In a series of experiments optimal conditions for the extraction of the radiation-induced fraction have been investigated. Two phosphate buffers pH 7.2-7.4 differing in molarity — 0.02 and 0.1 M were compared. In addition, a 0.1 M phosphate buffer pH 7.2-7.4 containing 2 % glycerol was also tried. Only extracts prepared with 0.1 M phosphate buffer contained the radiation-induced fraction, thus indicating that solubility may be a critical factor for extraction. An attempt was made therefore to study in a more direct way the effect of irradiation on the solubility of proteins. A method based on stepwise extraction of the proteins precipitated by saturated ammonium sulphate by a series of ammonium sulphate solutions of decreasing molarity was used for this purpose.

In experiments with four extraction steps most of the proteins were extracted with the 1 and 2 M ammonium sulphate solution, small amounts of material appeared in the 3 M and in the water extract. The 4 M extract contained no proteins, its high absorbancy being probably due to low molecular substances which on the basis of the high A_{260}/A_{280} ratio appear to be nucleic acid derivatives. In the pattern of extracted proteins striking differences were noted between the untreated and the irradiated samples (Figs. 9 and 10). Whereas qualitatively the patterns were very similar for all samples, there were marked quantitative differences in the content of all protein fractions. The greatest difference was found with the 5 Mrad sample (Fig. 9). The percentage content of the 2 M peak was reduced to 40-50 % of the corresponding peak of the untreated sample. Similarly the 1 M peak is reduced to about 50 % of the unirradiated control. Also for samples irradiated with the lower dose (1 Mrad) a distinct decrease of the 1 and 2 M peaks was observed. A small amount of material could always be extracted with the 3 M solution. Determination of the A_{410}/A_{280} ratio indicated that this fraction contains myoglobin. There was a great variation in the A_{280}/A_{260} ratio for different peaks and for different fractions within a single peak indicating differences in the proteins composition of these fractions. With the 5 Mrad sample the A_{280}/A_{260} ratio was consistently lower for all the fractions of the 1 M and 2 M peak than for the corresponding fractions of the untreated meat.

In an improved extraction procedure using seven instead of only four extraction steps basically the same results were obtained as described previously (Fig. 10). A strong decrease in solubility was observed on irradiation. The decrease of solubility was most evident with solutions of 2, 1.5 and 1 M molarity. In addition to changes of the absorbancy ratio we have analyzed the

fractions obtained on gradient salt extraction by thin-layer gel chromatography. For this purpose the proteins were precipitated from the extracts by raising the concentration of ammonium sulphate. The protein precipitate was centrifuged off, dissolved in buffer, dialyzed and concentrated to the desired protein concentration. Thin-layer gel chromatography of the 2 M extract revealed little difference between the untreated sample and the 5 Mrad sample. Marked differences were however noted in the 1 and 1.5 M extracts. There was a distinct shift to components with higher molecular weights in the irradiated samples. These results could be explained by assuming radiation-induced aggregation.

A — unirradiated



B — irradiated with 5 Mrad

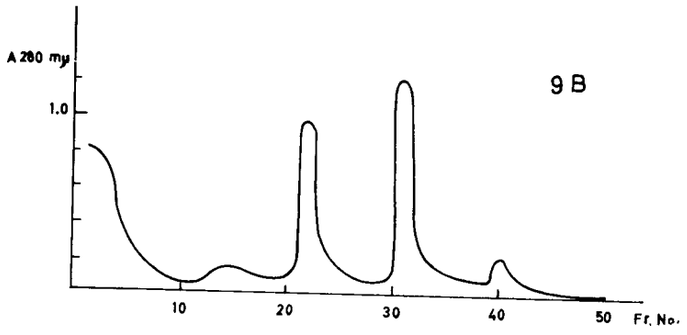
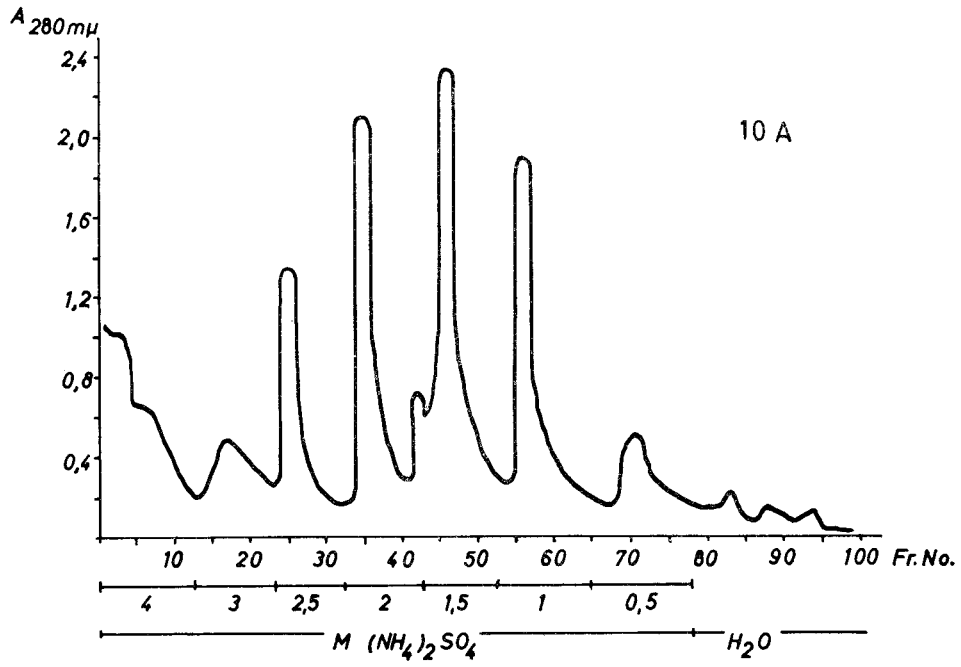


Fig. 9 — Gradient salt extraction with ammonium sulphate of beef (4 extraction steps).

A — unirradiated



B — irradiated with 5 Mrad

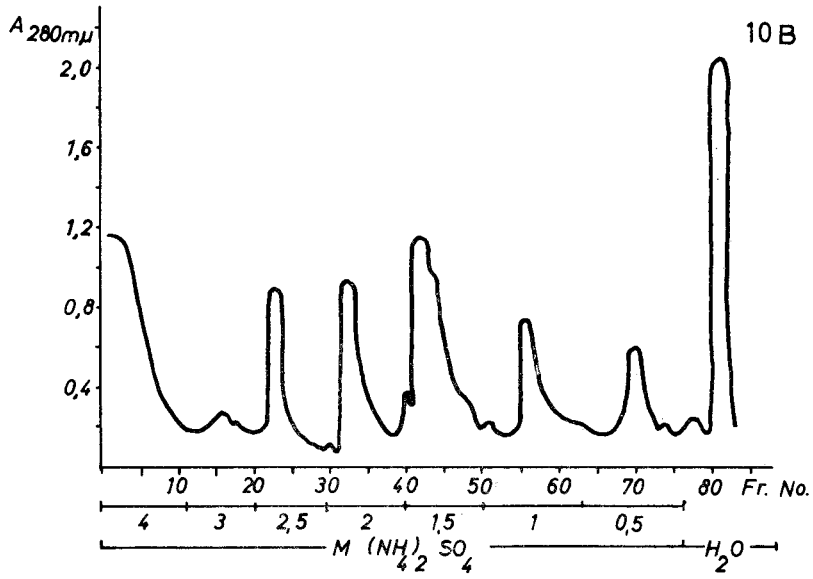


Fig. 10 — Gradient salt extraction with ammonium sulphate of beef (7 extraction steps).

Most of the techniques used for identification of irradiated food on the basis of changes in proteins require that the protein be obtained in soluble form (thin-layer gel chromatography, electrophoresis and immunoelectrophoresis, ion exchange chromatography). With meat and fish only 20-40 % of the total protein, the sarcoplasmic proteins can be extracted with solutions of low

ionic strength (16). The myofibrillar proteins, which amount tp 40-75 % of whole muscle proteins, can be extracted only with high ionic strength salt solutions. Radiation-induced changes of these proteins could not be studied by the above mentioned methods.

Salt extraction of an ammonium sulphate precipitate of the whole muscle proteins may be expected tc disclose differences both in the sarcoplasmic (myogen) and myofibrillar proteins (myosin). The results obtained indicate that on irradiation a considerable fraction of the muscle proteins is insolubilized. This insolubilization may be caused either by interaction of the sarcoplasmic and myofibrillar proteins or by interaction of these proteins with the insoluble stroma proteins. Radiation-induced changes in proteins in food products may differ considerably from effects observed on irradiation of protein solutions due to different physico-chemical conditions.

While changes of solubility of proteins could provide a promising approach to identification of irradiated meat the column extraction technique appears to be not enough attractive for this purpose since it is too laborious and not suitable for routine analysis. Therefore, most of our work was carried out by thin-layer gel chromatography, which in addition to the relative simplicity affords the advantage of simultaneous analysis on a single plate of the irradiated material and the control.

4 — SUMMARY

In beef and pork irradiated with a dose of 1 and 5 Mrad a new radiation-induced, sarcoplasmic protein fraction was observed on thin-layer gel chromatography on Sephadex G-200. Due to its high R_M value ($\sim$ 2.4) the radiation-induced fraction migrating ahead of all other sarcoplasmic protein fractions (R_M 1-2.1) of untreated meat could be readily identified in the chromatographic pattern. Irradiation experiments with isolated sarcoplasmic proteins strongly suggest that the radiation-induced fraction results from aggregation of these proteins.

The amount of the radiation-induced fraction depended on dose — it increased with increasing dose. At — 30 °C the amount of this fraction was smaller than at 0 °C. Dose rate was found to be another factor affecting the formation of the radiation-induced fraction.

Storage of meat irradiated with 5 Mrad at room temperature for periods up to 10 weeks has shown that the radiation-induced fraction is stable and is still present in the sarcoplasmic extract. Meat preserved at — 30 °C for comparable periods or subjected repeatedly to freezing and thawing cycles did not contain the $R_M \sim 2.4$ fraction. These results prove that the radiation-induced fraction is specific for irradiation.

Solubility studies based on stepwise extraction of a protein precipitate absorbed on an inert carrier with a series of ammonium sulphate solutions of decreasing molarity have shown that the fractions extracted with 1, 1.5 and 2 M ammonium sulphate are strongly reduced in irradiated meat. Solubility studies appear to be less suitable for routine analysis than thin-layer gel chromatography.

The demonstration of a radiation-induced, sarcoplasmic protein fraction can serve as a basis for identification of meat irradiated with a dose of 5 Mrad. At the lower dose of 1 Mrad identification depended on irradiation conditions. The detection of the radiation-induced fraction by thin-layer gel chromatography is relatively simple and suitable for routine analysis. Several samples of irradiated meat can be run simultaneously on a single plate and compared with the untreated control. The radiation-induced fraction appears to be specific for irradiation.

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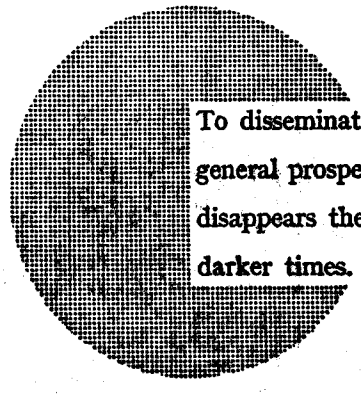

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Alfred Nobel

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