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Department of Defence
Defence Science and Technology Organisation
Armed Forces Food Science Establishment
Scottsdale, Tasmania

AFFSE REPORT 3/77

The Effects of Feeding Formaldehyde Treated Meat to Rats

J. R. CASLEY-SMITH

DEPARTMENT OF DEFENCE
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(6) # THE EFFECTS OF FEEDING FORMALDEHYDE STABILISED MEAT TO RATS. (U)

(10) J.R. CASLEY-SMITH

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S U M M A R Y

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It is therefore concluded that there is very little likelihood of meat, treated in such a manner, having any deleterious effect on men. (U)

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16. SUMMARY:

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It is therefore concluded that there is very little likelihood of meat, treated in such a manner, having any deleterious effect on men. (U)

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C O N T E N T S

THE EFFECTS OF FEEDING FORMALDEHYDE STABILISED MEAT TO RATS

by

J.R. CASLEY-SMITH

I N T R O D U C T I O N

The four previous reports (Casley-Smith, 1972; 1975; Casley-Smith and Ehmann, 1973; Casley-Smith et al., 1974) and a review (Casley-Smith, 1973) have covered the general background to this work and the results of experiments up to the end of 1975. The present report covers the final stage of this work which was the direct responsibility of the University of Adelaide, viz. a long-term feeding trial of the product in order to ensure its harmlessness.

The basic problem, outlined by Hutchinson (1970), was to improve the quality of freeze-dried steak (and other large pieces of meat) so that they would be acceptable on reconstitution and could thus be used in ration packs. While normal freeze-drying methods have been found to be very good on certain materials - especially those which come as small particles, they do not work well on large pieces of meat. These on reconstitution, tend to be tough, of lowered satiety value, and tend to remain wet on the outside and dry on the inside, with water being easily expressed from them on slight pressure (Dowman, 1971; Hutchinson, 1970; Venkata-Raman, 1971). While this would be of little consequence if the ration packs were only to be used for one or two days, it is considered that they must be suitable for use over periods of 5-7 days.

The food consumption habits and preferences of Australians indicate a desire to have slices or large pieces of meat available in the diet. Freeze-drying enables large pieces of various meats to be incorporated into combat ration packs. Thus research to improve the texture of reconstituted freeze-dried beef has an immediate practical goal. Alcoholic dehydration has been suggested also as an alternative to freeze dehydration (Casley-Smith et al 1974, Casley-Smith, 1975).

The underlying reasons for the difficulties with freeze-drying are unknown. They are likely to remain so until we have a much better understanding of the basic physics and chemistry of water in the tissues and how these are affected by this process and by reconstitution. However, it was considered (Casley-Smith, 1970) that it might be quite possible to find an ad hoc solution to the immediate problems by applying techniques of tissue stabilisation developed for electron microscopy. Here, it is imperative that the fine structure, and frequently the "fine physics and chemistry", of the tissue be preserved, with special emphasis on the cellular

membranes (Sjöstrand, 1967). It was suggested that the lack of stabilisation of these membranes might be the factor chiefly responsible for the difficulties experienced in freeze-drying (Casley-Smith, 1970). Relatively slow freezing rates have to be used, which are highly likely to cause multiple ruptures of many of the cellular membranes because of the formation of large ice crystals. Thus the contents of the cells would no longer be retained in their original compartments after reconstitution. They would be free to migrate from their normal sites so that the whole muscle would resemble a sponge containing meat soup. These suggestions have indeed been confirmed during this project. Electron microscopy has revealed that such ruptures do occur and that they are largely prevented by the appropriate pretreatment (Casley-Smith, 1973; Casley-Smith and Ehmann, 1973). The previous reports also show that such a pretreatment considerably improves the acceptability of the final product.

The pretreatment consists of fixing the tissues with low concentrations of formaldehyde and removing the unreacted reagent by washing in water which also includes phosphate buffers (pH 11) to increase the juiciness and water-holding-capacity (WCP) of the product. When preparing specimens for frozen-section electron microscopy, it is well known that such fixation renders the cells much less susceptible to ice-crystal damage, and probably more natural in appearance on reconstitution after drying following freezing (Sjöstrand, 1967; Bernhard and Leduc, 1967; Bernhard and Viron, 1971; Tokuyasu, 1973). The fixatives bind the proteins of the cells, and their membranes, together with co-valent bonds. It is thus much harder for ice-crystals to rupture cellular membranes and the osmotically-active constituents of the various compartments are much less likely to leave them on reconstitution. Hence water will tend to re-enter these compartments to extents roughly equivalent to the amounts present originally.

Formaldehyde has been used in our work for a number of reasons. It is known to penetrate the tissues very rapidly (Dempster, 1960; Hopwood, 1967, 1969). It is very effective in cross linking the proteins, producing the finest pores in the membranes of any of the conventional fixatives (Sjöstrand, 1967). These pores are quite distinct from those between the cells, upon whose width depends the rapidity of water re-entry during reconstitution - formaldehyde does not alter these intercellular pores (Casley-Smith, 1975). Formaldehyde does have the disadvantage of making the material tougher, but it has been shown that this can be kept to minor levels, which are not significant compared with its advantages (Casley-Smith, 1975; Casley-Smith et al., 1974). There was, however, one potentially most significant disadvantage - it might act as a poison. Certainly it gained an unsavoury reputation when it was used as a preservative by unscrupulous sausage manufacturers early in this century, but this was when it was simply used in high concentrations as a preservative, with no attempt to remove the unreacted reagent. In fact, it has been fed daily to sheep in relatively far greater doses than likely to be received by men eating our product, for periods of years, with no ill effects (Durand, 1971). Also, we have shown that meat

normally contains a small amount of formaldehyde, which actually exceeds the levels which remain after our treatment (Casley-Smith and Ehmann, 1973; Casley-Smith et al., 1974). Washing, cooking and freeze-drying remove the unreacted formaldehyde. The reacted formaldehyde is attached to the proteins, and is digested and used in the body just like any normal food without ill-effects, being converted almost entirely to water and carbon dioxide in a short time (Durand, 1971; Warner, 1972 - personal communication).

However, sheep are not men. Since the gut of a ruminant is indeed so different from that of an omnivore, it was decided to carry out a long-term feeding trial in rats before undertaking extensive testing in men.

E X P E R I M E N T A L

One hundred young (~150 g) male rats were randomly divided into two equal groups. They were placed, five to a cage, in air-conditioned, semi-sterile rooms. (The rats were specific-pathogen-free, of the Wistar strain.) Water and rat nuts (W. Charlick Pty. Ltd.) were given ad lib. Five grams of formaldehyde treated, or normal, meat were given to each rat for 5 days of the week. This regime continued for 12 months. Since the meat was cut into small (~1 cm^3) pieces, there was plenty for all and each animal received approximately the same amount. On the days when the meat was fed, the animals ate this at once, in preference to the rat nuts. Thus these latter supplemented the meat rather than the reverse, but the rats received about 50% of their nourishment from the nuts which provided the requisite vitamins and minerals.

The meat was all obtained from a 3 year-old Hereford steer, cut into ~1 cm^3 pieces and either left untreated, or treated with formaldehyde. For this latter, the pieces were placed in 0.3 g/100 ml formaldehyde in a buffer consisting of 0.274 g/100 ml $Na_4P_2O_7$ and 0.226 g/100 ml NaH_2PO_4, which gave pH 11. Unlike the meat for humans, no sucrose was added. The meat was fixed for 4 hours at 20°C. It was then washed in a second solution for 12 hours. (This was similar to the first, but the formaldehyde was omitted.) In the first case the volume of the solution was 10-20 times that of the meat; in the second it was 50 times. In order to ensure that the final product would contain more formaldehyde than normal, the cooking and freeze-drying were omitted. These processes normally reduce the residual amounts of formaldehyde to about 20% of the amount remaining after the washing; these former levels are about 25% of the normal amounts which occur in untreated meat. Thus the present level was ~2,000µg/g, in place of ~50 µg/g which is usually found after the whole processing. The amount of meat eaten by the rats was approximately equal to 1.25 kg of meat eaten by a 70 kg man each day of the week, but with a formaldehyde content 4 times greater than normal.

Various procedures were used to estimate the effects of the

treated meat on the rats. They were weighed, and the total weights and rates of growth were compared. Samples of the brain, liver, kidneys, stomach, ileum and skeletal muscle were taken and the amounts of formaldehyde in them were determined by the method developed earlier (Casley-Smith, 1972; Casley-Smith and Ehmann, 1973). Samples of these organs were also fixed in Zenker's fixative (which does not contain formaldehyde), processed for light microscopy, and examined. These were randomised, examined without the observer knowing to which group they belonged, and graded for normality.

R E S U L T S

Both groups appeared equally active and healthy. At the end of the 12 months the mean weight of the animals eating normal meat was 300.1 (5.14) g; that of those eating the treated meat was 294.2 (5.61) g. (The Standard Errors of the Means are given in brackets after the Means.) There are no significant differences between these two groups using the F or the t-tests. The growth gains over the 12 months were 149 (7.3) g for the untreated group and 145 (5.8) g for the treated one: again neither the F nor the t-tests are significant.

The results of the biochemical estimates of formaldehyde are shown below (in µg/g):

	Normal Meat		Formaldehyde Treated Meat	
Brain	823	(62)	934	(79)
Liver	1310	(82)	1260	(94)
Kidney	1270	(75)	1300	(83)
Stomach	1050	(67)	1110	(75)
Ileum	981	(74)	1300	(69)
Muscle	1000	(50)	950	(76)

With the exception of the ileum, where the t-test is very significant ($P<0.001$), none of the other t-tests and none of the F-tests are significant. Since the specimens are not washed in any way - except for a very brief rinse to remove the gross contents of the gut - it is highly likely that the increase found in the ileum was caused by residual formaldehyde from the ingested meat, rather than being actually present in the lining of the ileum. This did not appear in the stomach, perhaps because the meat was less finely divided here so that the wash removed all of it.

It is clear that eating the treated meat for a year did not cause significant increases in the amounts of residual formaldehyde in the various organs.

The histological examinations also revealed no significant differences between the two groups. In all cases the organs appeared normal. Since it was found that there was an increase in the numbers of small round cells in the gut of the sheep (Durand, 1971), a special search was made for signs of chronic inflammation. None were found.

C O N C L U S I O N S

It is evident that the rats suffered no ill effects from this diet. This is similar to the sheep which ate even larger relative amounts of formaldehyde for twice as long (Durand, 1971). Here, even the mild evidence of chronic inflammation of the gut, which was found in the sheep, was not observed. Thus formaldehyde treatment of meat, followed by cooking and freeze-drying, will give a product which can be continuously consumed by men for very long periods without ill effects.

Taste-panel results of the product have been favourable, it has been shown to be non-toxic and it is a process which is relatively simple and inexpensive.

A C K N O W L E D G E M E N T S

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