Glavind, J. & Tryding, N. (1960). *Acta physiol. scand.* **49**, 97.
Hendley, D. D., Mildvan, A. S., Reporter, M. C. & Strehler, B. L. (1963). *J. Geront.* **18**, 250.
Hochstein, P. & Ernster, L. (1964). *Ciba Fdn Cellular Injury, Symp.* p. 123.
Jacobs, E. E. & Sanadi, D. R. (1960). *J. biol. Chem.* **235**, 531.
Maier, V. P. & Tappel, A. L. (1959). *J. Am. Oil Chem. Soc.* **36**, 8.
Nishida, T. & Kummerow, F. A. (1959–60). *J. Lipid Res.* **1**, 450.
O'Brien, P. J. (1964). *Chemy Ind.* p. 1544.
Olcott, H. S. & Dolev, A. (1964). *Proc. Soc. exp. Biol. Med.* **114**, 820.
Schneider, W. C. (1948). *J. biol. Chem.* **176**, 259.
Tappel, A. L. (1962). *Lipids and their Oxidation*, p. 367. Westport: Avi Publishing Co. Inc.

Animal Nutrition, First Part

Chairman : PROFESSOR S. K. KON, CBE, DSc, PhD, FRIC, *National Institute for Research in Dairying, Shinfield, Reading*

Animal diseases associated with autoxidation of dietary fat

By C. A. GRANT, *Department of Pathology, Karolinska Hospital, Stockholm* 60, *Sweden*

The theme of this Symposium—'Nutritional and toxicity problems associated with fats'—is an important one for nutritionists and biochemists. It is also an important one for pathologists who encounter these problems in a particularly tangible form, as part of everyday work in diagnosing animal diseases and in the evaluation of responses of animals to experimental procedures.

The important naturally occurring animal diseases associated with dietary fat are those associated with autoxidation of dietary fat. Pigs present the major problems although other species of domestic animals and poultry as well as wild animals in captivity are involved at times. The position of young ruminants, lambs and calves, is ambiguous, as Blaxter (1962) has pointed out.

Dietary background

Our present and still fragmentary knowledge of the relation between naturally occurring animal diseases and autoxidation of dietary fat stems from what were at first laboratory curiosities in chicks—encephalomalacia (Pappenheimer & Goettsch, 1931) and 'exudative diathesis' (Dam & Glavind, 1939). Dam and his associates in the 1930's and 1940's established a connexion between these lesions, oxidative instability of unsaturated dietary fat, and tocopherol. The spectrum of what were originally experimental lesions has widened to include skeletal muscle degeneration, dietetic liver necrosis, 'yellow fat' and haemolysis as well as the original observations of sterility and foetal resorption, in a number of species of laboratory animals. On the dietary side of the equation, the selenium-containing Factor 3 of Schwarz has come into the picture (Schwarz & Folz, 1957).

The classical experimental diets used to induce these lesions included large amounts of unsaturated fat, usually cod-liver oil. During the 1950's, the diet based

on *Torula* yeast and introduced by Schwarz (1952) became popular. Dam, Nielsen, Prange & Søndergaard (1957), however, demonstrated that the yeast of this 'fat-free' diet contained about 5% total fatty acids with linoleic acid representing some 45%.

What bearing do these laboratory studies have on naturally occurring disease in animals? The problem of naturally occurring disease and autoxidation of dietary fat usually involves animals being fed on standard, well-established rations. The problem is quantitatively a pig problem and most of our experimental work has been done on pigs.

Conventional pig rations contain up to 80–85% grain, mainly oats and barley. Oats contain roughly 5% fat and barley about 2·5%. The fatty acid content of Swedish grain samples (Table 1) has been studied by Lindberg and his associates (Lindberg & Orstadius, 1961; Lindberg, Bingefors, Lannek & Tanhuanpää, 1964; Lindberg, Tanhuanpää, Nilsson & Wass, 1964).

Table 1. *Percentage fatty acid composition of samples of Swedish grain determined by gas–liquid chromatography*

	Fatty acid								
Grain	Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic	PFA* (%)	Total fatty acids† (%)
Barley U 37:									
Unripe 25.VII	0·9	24·0	0·3	1·3	13·4	49·4	10·9	1·73	2·9
Unripe 1.IX	1·1	21·8	0·8	1·9	16·6	50·7	7·1	1·15	2·0
Ripe 23.IX	0·9	22·8	0·4	1·3	13·0	53·6	7·9	1·39	2·3
Oats U 36:									
Unripe 25.VII	0·6	16·7	0·2	1·0	41·7	30·1	9·8	1·22	3·1
Unripe 1.IX	0·4	13·4	0·4	1·2	28·3	51·5	4·9	1·69	3·0
Ripe 22.IX	0·5	17·2	0·3	1·6	37·0	41·7	1·7	1·90	4·4

* Polyunsaturated fatty acids. Enzymic determination. Figures refer to dry substance.
† Calculated from PFA value and relative proportions of fatty acids.

This means, to use Lindberg's figures, that a pig weighing about 50 kg and fed on a conventional ration consumes from 100 to 200 g cereal fat a day. Cereal fat is unsaturated, but the fat of fully ripe and properly harvested and stored grain is oxidatively stable and can remain so for years (Thafvelin, 1960 *a*,*b*). These ideal conditions are not always attained in practice, at least not in Northern Europe nor, I expect, in the UK. Wet summers, the use of combine harvesters and dryers, and the necessity of using newly harvested grain in feed mixtures are some of the factors probably involved in disrupting the oxidative stability of cereal fat. These aspects have been studied by Thafvelin and his associates (Thafvelin, 1960 *a*,*b*; Thafvelin, Swahn & Erne, 1960; Swahn & Thafvelin, 1962).

The processes by which the unsaturated cereal fats attain, and under normal conditions maintain, oxidative stability are poorly understood and undoubtedly

Table 2. *Oxidative instability in whole grain to illustrate the pattern for 'new grain'. Peroxide values before and after* (80°, 22 *h*) *acceleration of autoxidation*

Date	Peroxide value (m-equiv./kg fat) Before	After
1958: October	52	49
	25	80
November	10	30
	0	135
December	40	205
	22	140
1959: January	0	150
	0	0
February	14	0
	0	0
April	0	0

complex. The course of the process can be followed, however, and a typical example taken from one of Thafvelin's papers is given in Table 2.

This concerns a sample of barley harvested in 1958. The oxidative stability of the fat was evaluated by determining the peroxide content by simple iodometric titration at intervals throughout the storage period before and after heating at 80° for 22 h.

There was little measurable formation of peroxides in the unheated samples during the storage period. Heating the grain induced lively peroxide formation, and this poor oxidative stability persisted throughout the autumn and early winter. Later on, stable conditions were attained. Table 2 illustrates the attainment of oxidative stability in the fat of grain harvested in 1958, a wet and cold year. The summer of the next year, 1959, was exceptionally warm and dry and cereal fat attained oxidative stability in the fields before harvesting.

Oxidative stability of cereal fat can also be influenced by other factors. Grinding the grain, for example, can produce the pattern illustrated in Table 3.

Table 3. *Oxidative instability in ground grain to illustrate the pattern for mechanically damaged grain. Peroxide values before and after acceleration* (80°, 22 *h*) *of autoxidation*

Date	Peroxide value (m-equiv./kg fat) Before	After
1958: October	42	50
November	35	65
	45	140
December	75	90
	180	65
1959: January	140	30
	110	35
February	140	15

These results, also taken from Thafvelin *et al.* (1960), are based on the same original sample of barley described in Table 2. This time, however, the grain had been ground before storage.

At the beginning of the storage period there was a moderate level of peroxides, and heating the sample resulted in a further increase. In other words, autoxidation

https://doi.org/10.1079/PNS19660006 Published online by Cambridge University Press

was taking place but had not yet attained its full potential. As time went on, the level of peroxides rose steeply, i.e. the induction period had ended. At the same time, accelerating autoxidation by heating the samples simply depressed the peroxide level.

The full cycle of events is illustrated by the results for a sample of ground barley, moistened, and placed in an incubator at 37° (Table 4).

Table 4. *Pattern of peroxide formation in ground barley with a high water content and incubated at* 37°

Period of incubation (h)	Peroxide value (m-equiv./kg fat)
4·75	0
6·75	68
8	146
11	78
13	47

The pattern which emerges from Tables 2–4 gives the general outline of oxidative instability in cereal fat. The tables also illustrate some types of practical feeding problems which can be encountered: newly harvested grain which has not yet attained oxidative stability but is readily peroxidizable (Table 2), mechanically damaged (ground) grain in which peroxidation readily occurs (Table 3), and exposure to warmth and moisture (Table 4).

These by no means exhaust the possibilities—microbiological enzymic processes for example. Furthermore, we have much to learn about how oxidative stability of dietary fat is affected by the processing of commercial rations and the presence of pro-oxidants and antioxidants in them.

Before turning to the diseases associated with oxidative instability of dietary fat, there is a point which needs to be emphasized. The peroxide level in dietary fat has proved to be useful in evaluating a feed responsible for naturally occurring disease and in predicting the outcome of feeding experiments. This should not be understood as implying that the ingested peroxides as such are responsible for the manifestations of disease. In fact, it is unlikely that ingested peroxides are absorbed (Holman & Greenberg, 1958; Glavind & Tryding, 1960), although György (1962) does not fully share this view. What is important is the oxidative instability of dietary fat, its peroxidizability (Dam, 1962; Harwitt, 1962). The two-phase peroxide determinations of the type illustrated in Tables 2 and 3 help in assessing this property.

Naturally occurring disease and oxidatively unstable dietary fat

To describe the association between naturally occurring disease in animals and oxidative instability of dietary fat I shall take dietetic microangiopathy (MAP) in pigs as my main example. This disease has been described and defined previously (Grant, 1961) and I shall not be concerned with details here except for the illustration of particular points.

MAP is only one of several pathological responses of pigs to oxidatively unstable dietary fat in the interrelationship between unstable fat, vitamin E and Factor 3

(selenium) activity. At present, MAP has been reported only in pigs but we hope to demonstrate it soon in laboratory animals. Still, MAP is a useful example of the associations between naturally occurring disease and oxidatively unstable dietary fat. MAP is fairly common and it occurs in many countries—to my personal knowledge from examination of tissues in the UK, Canada and Germany. It is also a clear-cut lesion and, with the appropriate technique, easily demonstrated.

Naturally occurring MAP could be associated with oxidatively unstable dietary fat, usually cereal fat, by two-phase estimation of peroxidizability in the feed consumed by the pigs. Cereals are not the only possible source of oxidatively unstable dietary fat. Commercial concentrates and complete feeds often contain fat of dubious quality. MAP is also common among pigs fed on restaurant scraps—a type of feeding which is notoriously difficult to evaluate.

The observations on the circumstances involved in naturally occurring MAP have been translated into experiments summarized in Table 5. Details of the original experiments have been published elsewhere (Grant, 1961).

Table 5. *Summary of experiments to induce dietetic microangiopathy (MAP) in pigs by feeding on oxidatively unstable fat*

Expt	Basal diet	Main source of dietary fat	Oxidative status of dietary fat	Supplement	MAP
S	Grain+ skim milk	Grain	Unheated, PV 0	—	(±)*
			After heating, PV 140 (cf. Table 2)	—	+
K	Grain+ skim milk	Grain	Unheated, PV 160	—	+
			After heating, PV 0 (cf. Table 3)	—	−
M 1	Grain+ skim milk	Maize oil	Unheated, PV 0	—	−
			After heating, PV 250 (cf. Table 4)	—	+
H	Semi-synthetic	Maize oil	After heating+O_2, PV 250–380	—	+
				DL-α-tocopheryl acetate	−
A,F	Semi-synthetic	Cod-liver oil	Low peroxide content, high peroxidizability	— Sodium selenite	

PV, peroxide value (m-equiv./kg fat).
*See below.

The results of the experiments in Table 5 outline what happens when pigs are given fat which through oxidative instability has acquired properties similar to those of the cereal fats in Tables 2 and 4.

To repeat a point made earlier, the fact that MAP occurred when the peroxide level was high does not necessarily mean that the ingested peroxides were directly responsible for the lesion. The high peroxide levels simply indicate that the anti-oxidation system of the dietary fat was no longer effective.

Expt S in Table 5 represents the 'new grain' pattern in Table 2, cereal fat with a low peroxide content but a high degree of peroxidizability, i.e. an easily disrupted antioxidation system. The slight focal myocardial scarring in the myocardium of one of five pigs given the unheated grain has been interpreted as a sign of healing after

an episode of MAP and, hence, as a sign of the potentiality of the grain sample, more fully realized after peroxidation had been accelerated by heating.

Expt K represents the results of giving cereal fat with properties similar to that in the sample described in Table 2, i.e. the antioxidative system has been disrupted to permit lively autoxidation.

Expt MI is essentially the demonstration that it is the properties of the dietary fat which induce MAP. To avoid misunderstanding, a prerequisite—a negative prerequisite—is that the other components of the diet do not contain effective amounts of protective substances—vitamin E and Factor 3-active selenium compounds. What we usually do is to assess the 'inertness' of the other dietary compounds by small-scale experiments using newly weaned rats and dietetic liver necrosis, or chicks and diathesis or encephalomalacia, as standards.

As would be expected from the dietary context, α-tocopherol and sodium selenite (for its Factor 3 activity) effectively prevented MAP.

Even if selenium (as Factor 3) prevents MAP, the mere absence of selenium is not the only factor involved in causing MAP. In these experiments, given the absence of effective amounts of Factor 3 and tocopherol activity, manipulation of the dietary fat was the decisive factor in determining the outcome. This means that the effect of sodium selenite could perhaps more appropriately be described as pharmacological rather than nutritional. With the small amounts of selenium used in our experiments—0·2 mg $Na_2SeO_3 \cdot 5H_2O$/kg feed—the selenium content in the tissues of the pigs did not reach toxic levels (Grant, Thafvelin & Christell, 1961). But selenium salts are highly toxic and no system is so foolproof that the risk of poisoning pigs and consumers could be eliminated if selenium salts were generally added to pig feeds and not limited to use in dealing with emergency situations. It is difficult to defend the general supplementation of pig feed when the purpose is to compensate for its poor quality—oxidatively unstable fat.

MAP is not the only naturally occurring pig disease associated with oxidatively unstable dietary fat. The lesion in pigs corresponding to dietetic liver necrosis in rats has been described by Obel (1953). This particular manifestation is apparently seldom seen outside Scandinavia. In our experiments with cereal fat, vegetable oil and cod-liver oil, the incidence of dietetic liver necrosis was higher and the changes more severe in pigs given cod-liver oil.

Skeletal muscle degeneration in pigs as in other species is also one of the tissue responses to the interrelation between oxidatively unstable dietary fat and vitamin E. Questions of definitions, criteria and the interpretation of this aetiologically non-distinctive lesion, however, make it an unsatisfactory general example of a naturally occurring lesion. Nor have biochemical studies given a guide. Reviews of this aspect can do little more than list an array of metabolic changes without a coherent pattern in the muscle tissue of animals on experimental diets (Gloor & Wiss, 1964).

The same considerations apply to muscle degeneration in calves and lambs. Unlike with pigs, muscle degeneration in ruminants can involve the myocardium primarily as well as skeletal muscles. In his account of vitamin E in relation to disease of cattle and sheep, Blaxter (1962) found it necessary to distinguish between the

experimental lesion, usually induced by artificial diets containing unsaturated fat, and the naturally occurring disease. The experimental lesion can be prevented by giving large amounts of vitamin E, or other antioxidants, but not by selenium salts. Naturally occurring muscular degeneration in ruminants, on the other hand, can at least be controlled by giving selenium salts in micro-amounts or by giving very large doses of vitamin E. At any rate, there is no clear-cut relation between dietary fat and normally occurring muscle degeneration in ruminants. Since the field is still open for suggestions, there is the possibility that the lesion is associated—whether primarily, secondarily, or concomitantly—with rumen dysfunction. In our autopsy material, lambs with muscular degeneration almost invariably have abnormal rumen contents judged by the admittedly inadequate criteria which can be applied after death—colour, consistency, and smell. If the rumen contents of lambs with muscle degeneration in other parts of the world are similarly abnormal, a follow-up might well prove worth while.

A glance at, for example, recent issues of the *Annual Review of Biochemistry* will reveal the disordered and fragmentary state of present knowledge and theory about the pathogenesis of the type of diseases considered here. Establishing the links in the chain from the ingestion of oxidatively unstable fat to the development of the full-blown lesions offers tantalizing problems for the joint efforts of nutritionists, biochemists and pathologists. These diseases also represent an economic problem and one which is likely to grow in step with the ever-increasing industrialization of the feeding and husbandry of domestic animals.

REFERENCES

Blaxter, K. L. (1962) *Vitams Horm.* **20**, 633.
Dam, H. (1962). *Vitams Horm.* **20**, 527
Dam, H. & Glavind, J. (1939). *Nature, Lond*, **143**, 810.
Dam, H., Nielsen, G. K., Prange, I. & Søndergaard, E. (1957). *Experientia*, **13**, 493.
Glavind, J. & Tryding, N. (1960). *Acta physiol. scand.* **49**, 97.
Gloor, U. & Wiss, O. (1964). *A. Rev. Biochem.* **33**, 313.
Grant, C. A. (1961). *Acta vet. scand.* **2**, Suppl. 3.
Grant, C. A., Thafvelin, B. & Christell, R. (1961). *Acta pharmac. toxi.* **18**, 285.
György, P. (1962). *Vitams Horm.* **20**, 599.
Holman, R. T. & Greenberg, S. I. (1958). *J. Am. Oil Chem. Soc.* **35**, 707.
Harwitt, M. K. (1962). *Vitams Horm.* **20**, 541.
Lindberg, P., Bingefors, S., Lannek, N. & Tanhuanpää, E. (1964). *Acta Agric. scand.* **14**, 1.
Lindberg, P. & Orstadius, K. (1961). *Acta vet. scand.* **2**, 1.
Lindberg, P., Tanhuanpää, E. Nilsson, G. & Wass, L. (1964). *Acta Agric. scand.* **14**, 297.
Obel, A.-L. (1953). *Acta path. microbiol. scand.* Suppl. 94.
Pappenheimer, A. M. & Goettsch, M. (1931). *J. exp. Med.* **53**, 11.
Schwarz, K. (1952). *Proc. Soc. exp. Biol. Med.* **80**, 319.
Schwarz, K. & Folz, C. M. (1957). *J. Am. chem. Soc.* **79**, 3292.
Swahn, O. & Thafvelin, B. (1962). *Vitams Horm.* **20**, 645.
Thafvelin, B. (1960*a*). *Medlemsbl. Sver. VetFörb.* **12**, 205.
Thafvelin, B. (1960*b*). *Nature, Lond.*, **188**, 1169.
Thafvelin, B., Swahn, O. & Erne, K. (1960). *Medlemsbl. Sver. VetFörb.* **12**, 8.