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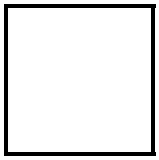

Histologic changes in mice fed with staple food*

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ABSTRACT

When mice were given staple food of seed quality corn and other cereals by subsistence farmers in Dhading and Bhaktapur areas in Nepal, histopathological changes were frequently found, particularly in the liver. All staple foods from Nepal were severely polluted with mould but only in one sample deoxynivalenol (DON) was found. (The most common liver changes, but to a lesser degree, were also found in the mice given seed quality corn with or without vitamin/mineral mixture.)

Kidney lesions such as tubulonephrosis, suspected parenchymatous degeneration, nonpurulent nephrosis or chronic pyelonephritis were found in mice given Nepalese corn and in the oats group.

In some groups there were histopathological findings like pronounced hyperkeratosis, papillary epithelial surface with hypergranulosis or uneven macropapillary surface observed in the distal part of the oesophagus and in the forestomach.

Because of the similar handling of nutrients for humans and mice, it is suggested that the results of this study be considered when nutritional surveys are made in less developed countries.

Keywords:

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* Produced by subsistence farmers in Nepal.

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INTRODUCTION

In many less developed countries the majority of the population is engaged in subsistence farming with limited facilities and little knowledge about how to store the produce properly. They largely live on the crops they produce with small means to buy additional food. In Nepal, the staple foods are mainly rice, corn, millet and wheat[1,2]. Even when pulses and legumes are grown, the quantities of this produce are limited by the size of the farming land. A survey in Nepal showed that about 88% of the total caloric intake of rural families consisted of cereal grains and that only 4.6% of the calories were fat[1]. Similar figures were found by a study from rural Bangladesh[3]. Large parts of the population in less developed countries may have a cereal intake of more than 90% of the calories, and rest of the calories is likely to come from sugar and/or root crops and not from high quality protein food[4].

The present study was undertaken because it seemed reasonable to assume that morphological changes in various tissues of experimental animals due to monotonous cereal diets could develop histopathological changes in liver and other tissues. Various studies have shown that the nutritional effects of various foods on mice and rats are surprisingly similar to those on humans[5,6]. Tissue changes in experimental animals might be of importance in explaining some morbidity patterns in poor rural families.

MATERIAL AND METHODS

Staple food from subsistence farmers in the Dhading and Bhaktapur areas of Nepal was collected and sent to the Institute of Nutrition in Vienna by air. Seed quality corn from Austria was also used in the trials.

Parts from each food sample were homogenised in peptone water and incubated at +23º C for 72 hours on Hygicult Y & R fungal slides (Orion Diagnostica, Espoo, Finland). If moderate or pronounced fungal growth was found, chemical analysis of possible presence of mycotoxins was made. Unfortunately, the amount of some samples was too small to make both a feeding trial and extensive mycotoxin determinations.

Nivalenol (NIV), deoxynivalenol (DON), ochratoxin A (OTA), cyclopiazonic acid (CPA) and Kojic acid were determined by the Bundesanstalt für Agrarbiologie, Linz, Austria. Aflatoxins and fumonisins were analysed by ELISA kits obtained from Neogen, Lansing, U.S.A.

The food samples were passed through a mill with 2-mm sieve. They were then thoroughly mixed with water as dough and boiled for 3 min. The dough was formed into flat cakes and then heated in a ventilated oven at +55º C for 24 hours. This cracker like "bread" was kept refrigerated at + 6º C until used.

Swiss OFI-SPF mice from the Experimental Animal Research Institute, University of Vienna, were used in the study. After weaning at 21-22 days of age the mice were kept individually in macrolon cages with wooden shavings. They were fed *ad libitum* with free access to tap water. The animal room had a temperature of +22º C with a 12 h light-dark cycle. The mice were usually weighed every other day. The general behaviour, stools and urine of the mice were also checked every other day. The

control mice were given commercial mouse feed (MF) of the same age as the test mice included.

After each feeding trial the mice were killed by decapitation. The time of killing was always between 10-12 AM. The gastrointestinal tract, liver and kidneys were fixed in buffered formalin. Before fixation, the liver and kidneys were weighed to determine the relative weights. The tissues were embedded in paraffin and the sections stained with hematoxilin-eosin. The two pathologists of the team made the microscopic examinations separately on coded slides.

RESULTS

All grain samples from Nepal had a moderate to heavy growth of different fungi and yeast between 10^5-10^6/ml. In one corn sample (I-17) 0.48 ppm of DON was found. Sample of the Millet I-15 with heavy mould and yeast growth had a very strong mouldy smell.

Signs of regenerative processes were observed in all the livers with histopathological changes. Table I shows that all livers of the mice fed on Austrian millet (AM) and millet from Nepal (I-15) had hydropic degeneration with varying degree of severity. The oesophagus of the mice receiving the mouldy I-15 diet had moderate to pronounced hyperkeratosis. The microscopic pictures of the kidneys were normal although one mice fed on Austrian millet (AM) showed a suspected parenchymatous degeneration. On the other hand, no such histopathological changes were observed in the control mice, fed on commercial mice fed (MF).

Table I: Microscopic findings in different tissues of mice fed with Austrian millet (AM), millet from Nepal (I-15) and commercial mice feed (MF).

The mice were 32 days old at start and given the different diets for 21 days. I-15 contained *Mucor raecemesus* 1x 10^5, *Aspergillus funigatus* 4 x 10^5 and *Asp. flavus* 3 x 10^3 but no mycotoxins were found.

0=normal; 1+= mild changes; 2+=moderate changes; 3+=pronounced changes, Wt. ch.= weight changes

Feed	*Wt. ch*	*Liver*	*Oesophagus*	*Kidney*
AM	3	1+Hydropic degeneration, Zone I, regeneration.	0	Suspected parachymatous degeneration
AM	3	1+Diffuse hydropic, 1+fat degeneration; regeneration	2+Hyperkeratosis	0
I 15	1	1+Diffuse hydroopic degeneration, regeneration	3+Hyperkeratosis	0
I 15	0	3+Hydropic degeneration, Zone II-III, regeneration	3+Hyperkeratosis	0
MF	9	0	0	0
MF	0	0	0	0
MF	6	0	0	0
MF	7	0	0	0

Table II shows that in the Rice II and Corn II groups all livers except one had hydropic degeneration, and that two of the mice in the Corn II group had mild nonpurulent inflammation in the pelvic region of the kidneys. In the Rice II group the oesophagus of one mouse was normal while the others had marked histopathological changes. On the other hand no such

histopathological abnormalities were found in the liver, kidneys and oesophagus of the control group mice fed on MF.

Table II: Microscopic findings in liver, kidney and oesophagus of mice given rice (Rice II) and corn (Corn II) from Nepal compared with commercial mice feed (MF). The age of the mice at start was 24 days and the trial lasted 24 days.

Feed	*Weight Change*	*Liver*	*Kidney*	*Oesophagus*
Rice II	2	2+Diffuse hydropic degeneration, luecocytic infiltration, regeneration	0	0
Rice II	5	1+Hydropic degeneration, Zone III, regeneration.	0	2+Hyperkeratosis, 3+papillary squamous epithelial surface with hypergranulosis
Rice II	5	0	0	2+Hyperkeratosis, 2+uneven micropapillary surface
Rice II	4	1+Diffuse hydropic degeneration, regeneration.	0	3+Hyperkeraosis, 3+papillary squamous epithelial surface with hypergranulosis
Corn II	-1	1+Diffuse hydropic degeneration, regeneration.	0	0
Corn II	6	2+Diffuse hydropic degeneration, regeneration	1+Nonpurulant inflammation. Pelvic region	0
Corn II	4	1+Diffuse hydropic degeneration, 1+Peripheral fat degeneration, eosinophilic inclusions, regeneration.	0	0
Corn II	-2	1+Diffuse hydropic degeneration, regeneration	1+Nonpurulant inflammation. Pelvic region	0
MF	5	0	0	0
MF	9	0	0	0
MF	8	0	0	0
MF	8	0	0	0

Table III shows that livers of all the mice fed on Austrian corn of good quality and corn from Nepal had hydropic degeneration along with other histopathological changes. In three kidneys of the mice fed on Nepalese corn showed suspected parenchymatous degeneration and mild tubulonephrosis including one-control mice. It should be noted that in all groups, except MF, the weight of the mice stopped or decreased.

Table III: Microscopic finding in different tissues of mice given various corns diets. The starting age of the mice was 48 days and the trial lasted for 20 days.

Corn A was Austrian corn of good quality. The corns from Nepal were numbered: I-17, I-6, I-2, I-12 and C II.

Analysis showed that I-17 contained *Asp.fimigatus* $4x10^5$, *Fusarium spp.* $5x10^5$, *Penicillium spp.* 12×10^5. The chemical analysis revealed 0.48 ppm DON. To the I-6 group, C II only was given the last 8 days, and to the I-12 group, C II for the last 7 days. The sex and weight differences (wt) are listed.

Feed	*Sex*	*wt.*	*Liver*	*Kidneys*	*rel wt.*
Corn A	M	-3	2+ Diffuse hydropic degeneration, eosinophilic inclusions, regeneration	0	
I-17	M	-3	2+ Diffuse hydropic degeneration, eosinophilic inclusions, regeneration 2+ Diffuse fat degeneration	0	2.1
I-6–CII	M	-3	2+ Hydropic degeneration, zone I, eosinophilic inclusions, 3+ Diffuse fat degeneration, regeneration	Suspected parenchymatous degeneration 1+ Tubulonephrosis	
I-6–CII	F	1	3+ Diffuse hydropic degeneration, eosinphilic inclusions, 3+ Diffuse fat degeneration, regeneration	1+ Tubulonephrosis	
I-2–CII	F	0	3+ Diffuse hydropic degeneration, focal necrosis, 1+ Small drop diffuse fat degeneration, regeneration	Suspected parenchymatous degeneration.	
1-2–CII	M	-1	3+ Diffuse hydropic degeneration, focal nonpurulent inflammation, regeneration	Suspected parenchymatous degeneration.	
I-12	F	1	2+ Diffuse hydropic degeneration, leucemic infiltration, 2+ Diffuse fat degeneration, regeneration	0	
MF	F	3	0	1+ Tubulonephrosis	2.0
MF	M	4	0	0	
MF	F	4	0	0	
MF	M	3	0	0	

No abnormal microscopically findings were observed in the oesophagus or in the stomach of the various groups, except a slightly uneven macropapillary surface in I-17.

Table IVA shows the results when seed quality corn was given to the mice for 40 days. The mice were 24 days old at the start and divided into 3 groups. Group 1 was given corn for 40 days, group 2 was fed corn for 20 days and then only commercial mouse feed (MF) for another 20 days, and group 3 was fed only on commercial mouse feed (MF) for 40 days. In the first group, all livers developed mild to moderate diffuse hydropic degeneration. In the second group, only two livers had mild diffuse hydropic degeneration. In the control group (MF), no such histological changes of the livers were observed.

Table IVB shows that a vitamin/ mineral/trace element mixture added to the seed quality corn diet did not prevent the pathological changes in the liver. The mice were 61 days old at the start and given corn for 21 days. One group was fed on corn only whereas another group was fed corn along with vitamin/mineral/trace element added mixture. No such histopathological changes were seen in the control group (MF).

Table IV: The histopathology of livers in mice given seed quality corn.

A - The mice were 24 days old at start and divided into 3 groups.
Group 1 was given corn for 40 days; Group 2 for 20 days and then only MF for 20 days; and Group 3 only MF for 40 days.

B - The mice were 61 days old at start and given corn for 21 days.
To the group Corn +, vitamin/mineral/trace element mixture was added.

	A		**B**	
Groups	*Feed*	*Changes in Liver*	*Feed*	*Changes in Liver*
Group - I	Corn	1+Diffuse hydropic degeneration	Corn	2+Vacuole degeneration
	Corn	2+Diffuse hydropic degeneration	Corn	1+Vacuole degeneration
	Corn	2+Diffuse hydropic degeneration	Corn	2+Diff. vacuole degeneration
	Corn	1+Diffuse hydropic degeneration	Corn	1+Vacuole degeneration
	Corn	1+Diffuse hydropic degeneration	Corn	1+Vacuole degeneration
	Corn	1+Diffuse hydropic degeneration	Corn	2+Diff. hydropic degeneration
	Corn	1+Diffuse hydropic degeneration		
	Corn	1+Diffuse hydropic degeneration	Corn +	2+Diff. hydropic degeneration
			Corn +	1+Vacuole degeneration
Group - II	Corn/MF	0	Corn +	2+Vacuole degeneration
	Corn/MF	0	Corn +	2+Vacuol./1+Hydropic degen.
	Corn/MF	1+Diffuse hydropic degeneration	Corn +	2+Diff. hydropic degeneration
	Corn/MF	0	Corn +	1+Vacuole degeneration
	Corn/MF	1+Diffuse hydropic degeneration	Corn +	1+Vacuole degeneration
	Corn/MF	0		
	Corn/MF	0	MF	0
	Corn/MF	0	MF	0
Group - III	MF	0		
	MF	0		
	MF	0		
	MF	0		
	MF	0		

In Table V the results of various feed mixture of cereals and beans from Nepal on various tissues in mice are shown. The mice were 29 days old with a feeding period of 27 days. The mouse group I-16 Corn-Soya received permanently 2/3 of corn and 1/3 of soya for a period of 27 days, while the other groups received the first mentioned feeds for 20 days and the second for 7 days. All animals had hydropic liver degeneration, either as the only lesion or together with other changes as seen in the table. Some groups had minor changes in the oesophagus, and kidneys while the Rice/ Corn groups had moderate to pronounced hyperkeratosis. The stomachs of all animals were microscopically normal. Mice of the control group receiving commercial mice feed (MF) did not develop any such histopathological changes in their organs.

Table V: The effect of different diets on various tissues in mice 29 days old with a feeding period of 27 days.

The last 7 days the diet changed as seen below. All grains were from Nepal. I-17 Corn contained 0.48 ppm DON. II 15 Rice was unpolished. Stopped or decreased weight was noted except in the control group (MF).

Feed	*Sex*	*wt.*	*Liver*	*Kidneys*	*Oesophagus*
I-16 Corn-Soya	M	-1	1+Diffuse hydropic degeneration, regeneration	0	0
I-16 Corn-Soya	M	-2	2+ Diffuse hydropic degeneration, regeneration	0	0
I-16 Corn-Soya	M	-2	2+ Diffuse hydropic degeneration, regeneration	0	0
II-13 Corn/I-16 Wheat	M	5	3+ Diffuse hydropic degene., 2+fat degene., 1+necrosis, regeneration	0	Uneven small papil. surface
II-13 Corn/I-16 Wheat	F	1	3+ Diffuse hydropic degene., 1+fat degene., regeneration	0	0
II-1 Corn/I-15 Millet	M	1	2+Diffuse hydropic degene., eosinophilic inclusions, 1+fat degene., regeneration	1+Tubulonephrosis	Uneven small papil. surface
II-1 Corn/1-15 Millet	F	0	3+Diffuse hydropic degene., 3+fat degeneration, regene.	Suspected parenchymatous degen.	0
II-2 Corn/I-17 Corn	M	0	1+Hydropic degene., Zone I-II, eosinoplilic inclusions, regeneration.	0	Uneven small papil. surface
II-2 Corn/I-17 Corn	M	0	3+Diffuse hydropic degene., 3+fat degene., regeneration.	0	2+Hyperkeratosis
II-15 Rice/I-17 Corn	M	-1	2+Diffuse hydropic degeneration, regeneration	0	2+Hyperkeratosis
II-15 Rice/I-17 Corn	F	3	3+Diffuse hydropic degeneration, regeneration	0	3+Hyperkeratosis
MF	M	8	1+fat degeneration.	0	0
MF	M	6	0	0	0
MF	M	9	0	0	0
MF	F	6	0	0	0

Table VI shows the results from mice fed with two types of oats. One of these (BO) had a natural pollution with various mycotoxins: DON 1.2 ppm, NIV 2.4 ppm, Fusarenon X 0.3 ppm, 3-acetyl-DON 0.7 ppm, HT 2 0.3 ppm, Sirpentreol 1.5 ppm, MAS 0.2 ppm, DAS 0.1 ppm, and T2 0.2 ppm. The second group of oats had no mycotoxins and of good quality (GO). The GO oats were made isoenergetic with the BO by adding starch. The histopathological liver lesions of the oat groups show a variety of kidney lesions with elevated relative weights. Microscopic oesophageal lesions were also very common, and in some cases the forestomach was also affected. As reference one group of mice was given commercial mice feed (MF).

Table VI: Histopathological findings in mice fed with good quality oats (GO) and oats with natural pollution with various mycotoxins (BO).

The mice were 25 days old at start and the trial continues for 14 days (GO & BO trial). GO-MF-GO & BO-MF-BO trial was made on 25 days old mice, where GO or BO was given for 14 days after which MF was given for 30 days, and then GO or BO was fed for another 65 days. Abnormally high relative weights (rel wt) of the kidneys are shown. Only relative weights at 1.8 and over is listed. The weight change (Wt ch) is also given. *Indicates placid mice.

Feed	*Liver*	*Kidneys*	*rel wt*	*Sex ch*	*wt.*
GO	2+Diffuse hydropic degen., regen., 2+Small drop fat degen	0		M	2
GO	0	1+Non-purulent focal inflam	2.2	F	3
BO	0	1+Tubulonephrosis, suspected parencym. degen.	2.4	M	-2*
BO	0	1+Tubulonephrosis	2.2	M	-3*
BO	0	1+Focal lymphocytary inflamation	2.5	M	-3
BO	0	0		F	-6*
GO-MF-GO	2+Diffuse hydropic degen., regen., 3+Fat degen.	1+Chronic pyleonephritis		F	-1/2/7
GO-MF-GO	3+Diffuse hydropic degen., eosinophilic inclusions, regen. 3+Fat degen.	1+Chronic pylronephritis	2.0	M	0/10/0
GO-MF-GO	3+Diffuse hydropic degen., regen.	1+Chronic pylenephritis	1.8	M	2/13/1
BO-MF-BO	3+Diffuse hydropic degen., regen.	1+Chronic pylenephritis	2.0	M	-7/20/-2*
BO-MF-BO	0	3+Non-purulent nephrosis, 1+fat nephrosis	2.4	M	-3/14/-2*
MF	0	0		M 14 days	9
MF	0	0		M 134 d.	24
MF	0	0		M 134 d.	27
MF	1+Diffuse hydropic degen., regen.	0		F 14 d.	8
MF	0	0		F 134 d.	18

Table VI *(Contd...)*

Feed	*Oesophagus*	*Stomach*
GO	Uneven small papil. Surface	0
GO	2+Hyperkeratosis	2+Hyperkeratosis
BO	Uneven small papil surface	Infiltration of neutrophils and lymphocytes in lamin. propriae
BO	2+Hyperkeratosis, uneven micropapil. Surface	0
BO	2+Hyperkeratosis, uneven macropapil. durface	2+Hyperkeratosis
BO	Papil. squamous epithelial hyperplasis, 3+hyperkeratosis and hypergranulation	3+Hyperkeratosis
GO-MF-GO	Uneven, small papil. Surface	0
GO-MF-GO	Papil. squamous epithelial hyperplasia and hypergranulation	0
GO-MF-GO	0	0
BO-MF-BO	Uneven small papil. surface, 2+hyperderatosis	3+Hyperkeratosis
BO-MF-BO	Papil.squamous epithelial hyperplasis and hypergranulation	0
MF	0	0
MF	0	0
MF	0	0
MF	Uneven small papil. surface	0
MF	0	0

Table VII shows the effect of chicken feed (CF), naturally polluted chicken feed (OTA), chicken feed with added 1.50 mg. DON and 0.45 mg. 3-Ac-NIV (DON/NIV) and commercial mouse feed (MF) in mice. The high incidence of lesions in liver and oesophagus of the mice compared with the control group should be noted. In three cases abnormally enlarged uterus were observed.

Table VII: The effect of chicken feed (CF), naturally polluted chicken feed (OTA), chicken feed with added 1.50 mg. DON and 0.45 mg. 3-Ac-NIV (DON/NIV) and commercial mouse feed (MF) in mice which were 30 days old and fed the various feeds for 30 days.

The chicken feed (CF) contained added 0.08% L-lysine.HCl and 0.17% DL-menthionine.

It should be noted that all mice in the experimental groups, in spite of the histopathological changes, increases in weight.

Feed	*Sex*	*wt.*	*Liver*	*rel.wt*	*Kidneys*	*rel. wt.*	*Oesophagus*
OTA	M	5	1+Hydropic degen. Zone I, regen.	6.9	0	1.8	3+Hyperkeratosis, uneven macropapil. surface
OTA	F	5	1+Hydropic degen. Zone III, regen.		0		Uneven small papil. surface
OTA	F	4*	1+Hydropic degen. Zone I, regen.		0		3+Hyperkeratosis, uneven macropapil. surface
DON/NIV	M	8	1+Hydropic degen. Zone I, regen.	8.8	0	2.2	Uneven small papil. surface
DON/NIV	F	2	1+Hydropic degen. Zone I, 1+central fat degen.	7.3	0		2+Hyperkeratosis, uneven small papil. surface
DON/NIV	F	3	0		2+Chronic pyelonephritis		3+Hyperkeratosis, unevenmacropapil. surface
CF	F	3*	1+Hydropic degen. Zone I, regen.		Suspected parenchy., degen.		Uneven small papil. surface
CF	F	5*	2+Diffuse hydropic degen., regen.		0		Uneven small papil. surface
CF	F	5	1+Hydropic degen. Zone I, regen.		Suspected parenchymatous degen.		Uneven small papil. surface
MF	F	6	0		0		0
MF	M	7	0		0		0
MF	F	5	0		0		0
MF	M	6	0		0		0

* Abnormally large uterus

DISCUSSION

The present study shows that not only the various staple foods from subsistence farmers in Nepal but also seed quality produced morphological changes in the liver, kidneys, oesophagus and in some cases in the forestomach when given to the mice, regardless if the corn was without or with added vitamins/minerals/trace elements. As expected, the growth of the mice on staple food was generally retarded.

Cereals contain too little of certain essential amino acids, particularly lysine. A cereal diet without supplementation of higher quality protein compensating for the lack of essential amino acids is known to result in slower growth in domestic and experimental animals, sometimes accompanied by morphological and enzymatic changes in the liver[8,7]. In addition, poor storage of grain with high humidity and elevated temperature often results in mould growth, with or without mycotoxin production. Higher water contents in the grain initiates sprouting and also induces chemical reactions with a decrease in the biological value of the proteins, often called the Maillard reaction[8]. The harvested cereal grains can start to sprout at a temperature around +30^{o} C if the water content exceeds 15%[9]. This initiates enzymatic activities thus further increasing the temperature in the grain so that the protein biological value decrease[9,10]. As mentioned, all cereals from the farmers in Nepal had a pronounced growth of yeast and fungi with or without mycotoxin production, and in these groups the liver damage was more severe.

The profile of the essential amino acids varies in cereal grains. But a common feature, as mentioned, is that not only the relative amount of lysine but also other essential amino acids is too low[10,11,12,13]. In less developed countries, poor storage of the produce leads to deterioration of the cereal grains due to high humidity and temperature. At the same time the action of micro-organisms and insects is also very common. Mould growth usually does not take place in a dry condition when moisture is below 15%. For example, corn with moisture content of 13-13.5% is regarded as safe for the long-term storage. It is important to note that aflatoxin production can increase from 200 ppb to 2,500 ppb in only 3 days when field-harvested corn is stored at high moisture[14].

The fat in poorly stored grains easily becomes rancid (autooxidized). The higher temperature facilitates aldehyde groups (CHO) to react with the E-amino group in lysine[12,15]. Enzymes or gastric juice cannot split this binding. It has also been suggested that the Maillard reaction, except for a reduced digestibility and biological value, may also produce toxic substance and/or metabolic inhibitors[15].

All grain samples from Nepal had moderate to heavy fungal growth but only one sample had a measurable amount of DON (0.48 ppm), as seen in Table V. However, it is possible that mycotoxins have been present in other groups but in so low amounts that they were not detected by the analysis and perhaps synergistically could cause the histopathologic changes. In this connection, there is plenty of evidence that a low nutritional state increases the susceptibility to mycotoxins both in animals and in man[16,17].

Table VI shows the results after feeding mice with good quality oats and oats where a number of mycotoxins were present. Almost all animals, both GO and BO groups had kidney lesions, while moderate to profound liver lesions were particularly common in the GO group. In this group the oats was made isoenergetic with the BO oats by adding starch. During the "baking" of this feed, condensation products, as mentioned above, must have been formed adding to the severeness of the changes. The oesophagus and partly, also the forestomach showed morphological changes.

Making bread usually involves baking cereals with yeast. This process decreases the available lysine[18]. However, a study from India showed that unleavened thin wheat bread, "chapaties", which are baked for a short time, only lost 4% of added lysine while 25% was lost in leavened bread[19]. This is of importance to note in view of the often-monotonous diet in poorer areas of the developing world. The importance to know the mechanism involved with protein quality is verified by a study where protein fortified biscuits were found to have a low protein biological value due to the Maillard effect[20]. Children eating enough of these biscuits may increase in weight, but this is then due to an increase of the fat compartment of the body[5,21].

The histopathological liver results were somewhat surprising because previous studies had shown that fat degeneration was only found when experimental animals were given rice, cassava, corn-meal and wheat flour[22,23]. The histopathological changes in the livers with various degrees of hydropic degeneration in the present study are actually rather similar to those described in the early stage of carbon tetrachloride poisoning with hydropic degeneration and necrotic development[24]. That study also showed that the vacuoles found in the hepatocyte did not consist of glycogen but it maybe present in the hydropic cells. About twenty years ago it was suggested that a hepatocellular carcinoma in experimental animals has a sequentially rather constant development where the first stage shows focal areas of hydropic degeneration[25]. In this case aflatoxin B 1 was used as the provoking toxic agent. In the present study, the experiment with cereal feeding was of comparatively short duration, and it is logical to assume that a longer feeding period would make the histopathological changes more severe. It should be emphasised that in none of the trials did spontaneous deaths occur. Only in the oats trials (Table VI), certain placidity was observed in the mice belonging to the BO group.

Irregular quantity and quality of food consumption is a common nutritional pattern in many developing countries. Periods of liberal food supply due to a good harvest or festivals therefore often alternate with severe food storage. A Moroccan study in rats tried to mimic this pattern[26]. It was then found that discontinuity of protein intake led to a decrease of its value as a complement for a poor basic diet. The liver DNA increased when protein supplementation was given with longer intervals, and at the same time the RNA decreased. This in turn leads to lower protein synthesis, particularly in the hepatocytes and facilitates hydropic degeneration. Hydropic degeneration of the liver has previously been associated with toxic agents and eosinophilic inclusions. Interestingly, the liver DNA in hypophysectomized rats and rabbits is much increased accompanied by splanchnimicria with a simultaneous loss of muscle and liver protein[27,28]. In rats given a lysine-poor diet not only showed a loss of liver, pancreatic and muscle protein, but also showed a loss of protein in the epididyml adidymal adipose cells[29]. The predictable series of morphological liver changes from vacuolary – hydropic degeneration to nodular hyperplasia and finally hepatocellular carcinoma seem to be similar in both humans and in small experimental animals[25]. Tables I, III, V and VI show that in some groups pronounced hydropic degeneration and fat degeneration developed after rather short trials. Further eosinophilic inclusions were particularly common in some groups fed with Nepalese corn.

The results of feeding mice with corn from Nepal and seed quality corn without any fungal growth are of interest in comparison to commercial mouse feed. It shows that all livers in the corn groups of

Nepalese origins and of seed quality corn had various degrees of diffused hydropic degeneration. Similar changes were found in three out of four mice in the rice group, whereas the livers of the control group appeared normal on microscopic examination.

The results of the present study indicate that the histopathologic changes in the liver probably is due to the poor protein biological value of the cereal grains used as mice feed and not necessarily due to the presence of fungi/mycotoxins. It is known that low protein diet makes rats more sensitive to both the acutely toxic and carcinogenic effects of afltoxin but also that mycotoxins increase the protein requirement of animals[16]. This may explain why, as seen in Table III, all mice on corn from Nepal had more severe liver lesions than the controlled high quality corn (Table IV). Other studies have shown that a low nutritional state increase the susceptibility to mycotoxins both in animals and man[17,30]. In those studies the protein biological value was high but the amount restricted. Corn is generally known to have low contents of particularly lysine and trytophan. It is evident that even seed quality corn will produce pathological liver changes, though milder than those produced by the Nepalese corn when given to the mice. The supplement of the seed quality corn with vitamin/mineral/trace element mixture did not improve the liver histopathology (Table IV b).

The histopathological changes in the oesophagus and forestomach of mice are difficult to evaluate. It was found that the most accurate evaluation should be made on slides showing the most distal part of the oesophagus and stomach should be regarded as preliminary and not entirely conclusive at this stage because of the relatively short feeding periods in the various groups. One of the studies has shown that when rats were given a diet low in protein, there was hyperplasia with thickening and formation of ridges of the squamous epithelium of the forestomach[31]. Also, it was shown that there is a high correlation rate of oesopahgus cancer in Transkei, South Africa, and the home-grown corn polluted with *F.moliniforme*. When rats were given this type of corn for a period of more than a year, they developed primary liver cancer and oesophagus basal cell hyperplasia[32]. The oesophagus and forestomach of mice were lined by the same epithelium as in rats. In mice where T-2 toxin was incorporated in semi-purified feed at different protein levels, gastric hyperkeratosis was observed[30]. In this study the hyperkeratosis occurred at all protein levels. A number of disease with morphological tissue changes have been attributed to fumonisin B1, among them oesophageal cancer is one[33,34]. In weaning pigs given fumonisin B1hyperplastic plaques, hyperkeratosis and formation of papillary down growths in the distal oesophagus mucosa were found[35]. Also mycotoxins produced by *Alternaria alternata* have been implicated with this type of cancer, as shown by a study from China[36]. Ulcer and cell infiltration in the forestomach of mice have been observed after high oral doses of DON and NIV[37]. In young pigs, low level exposure to *Fusarium* mycotoxins like DON, results in changes of certain immunological and haemotological parameters, but changes occur also in the oesophageal region of the stomach and oesophagus with thicker wall and higher degree of folding[38]. It is likely that the histopathological changes observed in the oesophagus and in the forestomach would have become more severe, had the trials been of longer duration.

CONCLUSION

In less developing countries like Nepal, poor storage of food grains results not only in mould growth, with or without mycotoxin production, but decrease in the biological value of proteins. Regular consumption of cereals or diet having low biological value protein leads to deterioration or slow growth of tissues leading to malnutrition. The term protein malnutrition is often used in the literature and usually means a too low total protein intake. Thus, the use of the term protein energy malnutrition (PEM) is

confusing and often wrongly interpreted. Rather, in many of the less developed countries it would be more appropriate to talk about protein quality malnutrition, as shown by the results of the present study.

Consumption of monotonous cereal diet, with or without polluted by mycotoxins, could produce severe histopathological changes in liver, kidney and oesophagus. It is also important to note that supplementing with vitamins/minerals/trace elements in the diet could not prevent such pathological changes. This might be of importance to explain some of the morbidity patterns in poor rural families in less developing countries like Nepal.

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