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The use of short tests for mutagenicity for the evaluation of any possible genotoxicity in animal-based foodstuffs, with particular reference to meat and meat products.

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Control and sanitary Inspectors of animal-foodstuffs no doubt feel the need to have at their disposal methods of analysis capable of detecting the total possible toxicity in foodstuffs (7). With the wide-spread use of short tests for mutagenicity, it has been possible to solve the problems concerning genotoxicity in this field (11).
As a result genotoxic substances have been divided into two groups: 1) substances responsible for genotoxicity which find their way into foodstuffs by chance as a consequence of environmental pollution and various animal treatments (heavy metals, pesticides, drugs) and 2) substances whose genotoxicity results from voluntary, even if indispensible, operations such as the addition of chemical substances (additives) and heat and radiation treatment (active radicals, amino-acids pyrolizates etc.) (3-7-9-11).
In reference to the latter group, any possible genotoxicity can be evaluated as s single occurrance resulting from the particular treatment or substance added.
However, in the former group given the unpredictability of the occurrence of contamination, the evaluation of genotoxicity should be made for each sample. Since it is pratically impossible to carry out such detailed evaluation, it is worthwhile to asses the conditions of contamination of the entire food-producing stock by evaluating the presence of mutagenicity in milk and urine samples (10). Naturally these enquiries must be considered complementary to those of a clinical (sterility, abortion, neo-natal deformations) and anatomo-pathological nature.
In keeping with the principles stated above, we have utilized for some time now the "Salmonella/microsome test" to asses the mutagenic activity due to particular treatments and to indicate the presence of active substances in food-producing animals through the

detection of mutagenicity in milk and urine samples.

PERSONAL RESEARCH

EVALUATION OF THE TOTAL GENOTOXICITY IN ADDITIVES AND FINISHED FOOD PRODUCTS.

Materials and methods.

5 samples of violet crystal inks used for sanitary marking of meats, 8 samples of natural aromas, 17 samples of smoked cheeses, 10 samples of no smoked cheeses, 15 samples of broth cubes, 5 samples of meat extracts, 54 samples of cooked dressed pork products, 46 samples of meat or fish based conserve, 22 samples of milk, both of long-term and short-term preservation;
nitrosation of natural aromas carried out according to Marquardt et Al. (6); extraction of any mutagenicity in cheeses and natural aromas with and without nitrosation carried out according to Marquardt et Al. (6), for the meat extracts and broth cubes according to Commoner et Al. (3), for the meat based products according to Felton et Al. (4) and for the milk according to Green et Al. (5); the evaluation of the cytotoxicity of the sample was carried out according to Felton et Al. (4);
evaluation of mutagenicity carried out with the "Salmonella/microsome test" according to Ames et Al. (1), with and without microsomal activation (S-9) using the strains TA1535, TA1537, TA98, TA100, testing for each plate, up to 20 ug of dry ink substance, the equivalent of 1 g of natural aromas, up to 12 g of cheese, up to 3 g of broth cubes and 600 mg of meat extracts, the equivalent of 40 g of meat and fish conserves and the equivalent of 20 ml of milk; (for each sample, 2 plates were set up and the experimet was carried out twice).

Results.

From table 1 we can see that 12 samples of smoked cheeses, 10 samples of broth cubes and all the samples of meat extracts caused a significant increase of revertans in relation to the controls, the first on the TA1535 strain without microsomal activation and the others on the TA98 strain with microsomal activation.

Table 1: valutation of mutagenic activity of additives and finished food products carried out by "Salmonella/microsome test".

PRODUCTS	N°	USED STRAINS TA1535	TA1537	TA98	TA100	AMOUNT PER PLATE	N° SAMPLES POSITIVE	STRAIN ACTIVE (*)	N° REVERTANTS (N° control)
SANITARY INKS	5	+	+	+	+	20 ug	-		
NATURAL AROMAS	8	+	+	+	+	1 g	-		
after nitrosation	8	+	+	+	+	1 g	-		
SMOKED CHEESES	17	+	+	+	+	12 g	12	TA1535-	160(15)
NO SMOKED CHEESES	10	+	+	+	+	12 g	-		
BROTH CUBES	15	+	+	+	+	3 g	10	TA98 +	630(45)
MEAT EXTRACTS	5	+	+	+	+	600 mg	5	TA98 +	915(45)
COOKED DRESSED PORK									
mortadelle	18			+		40 g	-		
cooked hams	16			+		40 g	-		
zampone-cotechino	20			+		40 g	-		
CANNED MEAT	34			+		40 g	-		
CANNED FISH	12			+		40 g	-		
MILK									
long-term pres.	12			+		20 ml	-		
short-term pres.	10			+		20 ml	-		

(*) - Means without microsomal activation.
+ Means with microsomal activation.

The concentrations of samples tested did not provoke cytotoxicity.

Conclusions.

Of all the examined samples of foodstuffs or products which can be utilized for foodstuffs, that undergo sanitary checks, only a few smoked cheeses, the broth cubes and the meat extracts exhibited mutagenic activity. In particular it is to be noted that the heat treatment of some typical meat products of the local dressed pork and conserve industry does not provoke the neo-formation of mutagenic substances.

DETECTION OF GENOTOXICITY IN THE URINE AND MILK OF CATTLE.

Materials and methods.

60 urine samples added with, as we can see from table 2, sodium azide, aflatoxine B1, 2-aminofluorene and 5'nitrofurfurilidene-4-idrossibenzidrazide (NFBI); 20 samples of urine taken from animals bred in Umbria; 60 milk samples added as above (see table 2);
separation of any possible conjugated mutagenic substances in the urine samples of animals bred carried out according to Commoner et AL. (2); concentration of the urine added by sodium azide obtained with the use of a rotating evaporator at temperatures below 37°C up to 20 times; extraction of mutagenicity contents carried out fot the others milk and urine samples according to Yamasaki et Al. (12); evaluation of the cytotoxicity of the samples, according to Felton et Al. (4);
evaluation of mutagenicity carried out with the "Salmonella/microsome test" according to Ames et Al. (1) with and without microsomal activation using the strains TA1535, TA1537, TA98, TA100, testing for each plate 7 ml milk an urine (for each sample, 2 plates were set up and the experiment carried out twice).

Results.

From table 2 we can see how a significant increase of the revertans with respect to the controls is caused by the samples obtained by concentrating urine added with 0.1 ppm of sodium azide, by the samples obtained according to Yamasaki et Al. (12) by urine

Table 2: valuation of mutagenic activity of samples of urine and milk added with mutagenic substances carried out with "Salmonella/microsome test".

SAMPLE	MUTAGEN ADDED	ppm	N°	AMOUNT PER PLATE, ml	STRAIN USED(*)	N° REVERTANTS	N° CONTROLS
URINE	sodium azide	0.01	5	7	TA1535 -	25	15
		0.1	5	7	"	62	15
		1	5	7	"	550	15
URINE	2-aminofluor.	0.01	5	7	TA98 +	40	35
		0.1	5	7	"	180	35
		1	5	7	"	262	35
URINE	NFBI	0.01	5	7	TA100 -	153	126
		0.1	5	7	"	306	126
		1	5	7	"	1218	126
URINE	aflatoxin B1	0.01	5	7	TA98 +	60	35
		0.1	5	7	"	237	35
		1	5	7	"	1185	35
MILK	sodium azide	0.01	5	7	TA1535 -	17	15
		0.1	5	7	"	22	15
		1	5	7	"	40	15
MILK	2-aminofluor.	0.01	5	7	TA98 +	55	35
		0.1	5	7	"	105	35
		1	5	7	"	454	35
MILK	NFBI	0.01	5	7	TA100 -	128	126
		0.1	5	7	"	290	126
		1	5	7	"	1115	126
MILK	aflatoxin B1	0.001	5	7	TA98 +	80	35
		0.01	5	7	"	204	35
		0.1	5	7	"	450	35

(*) - Means without microsomal activation; + Means with microsomal activation.

added with 0.1 ppm of 2-aminofluorene, aflatoxine B1 and NFBI, by the samples of milk added with 0.01 ppm of aflatoxine B1, with 0.1 ppm of 2-aminofluorene and NFBI and with 1 ppm of sodium azide.
The urine taken from cattle bred in Umbria did not provoke a significant increase of the revertants even after the freeing of any possible conjugated substances.
The concentration of samples tested did not provoke cytotoxicity.

Conclusions.

The "Salmonella/microsome test" which permits the detection of very low levels of genotoxic substances in milk and urine is a valid instrument for the detection of these substances in food-producing animals. Till now mutagenic activity has not shown up in the urine of cattle reared in Umbria. Further tests are being carried out, extending frequency and number of sampling in order to contribute to the knowledge of the extent of chemical contamination in this region of Italy.

Summary.

With the utilization of "Salmonella/microsome test" the Authors tested samples of additives and finished food products. They found mutagenic activity in some smoked cheeses, in broth cubes and meat extracts.
The utilization of "Salmonella/microsome test" also permits the detection of very low levels of genotoxic substances in milk and urine.

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