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TOXICOLOGICAL STUDIES ON SPIRULINA ALGA SOSA TEXCOCO S.A. PILOT PLANT FOR THE PRODUCTION OF PROTEIN FROM SPIRULINA ALGA*

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MEXICO

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* This document has been translated from an unedited original.

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C O N T E N T S

INTRODUCTION

Micro-organisms, including yeasts, fungi, bacteria, and the unicellular algae, offer interesting features as non-conventional sources of protein (Dabbah, 1970); (Lipinsky and Litchfield, 1970) which could contribute towards solving the need for food products which is becoming more urgent, particularly in the developing countries.

The use of algae as food for humans has been known for some centuries, forming part of the diet of the inhabitants of East Asia and Central Africa (Venkartaram et al. 1977) and of Mexico (Farrar 1966).

In the decades which followed the first World War investigators from several countries were interested in the cultivation of eucarpic green algae such as Chlorella and Scenedesmus which are capable of developing in simple cultivation media (Burlew 1953, Richard et al. 1961; Powell et al. 1961).

The various studies carried out with microscopic green algae, as proteins which use very wide ranges of substrates for their cultivation, have made it possible in some cases to select them on their potential for adaptation as human and animal feeding stuffs, based on the characteristics of their protein content, their good digestibility and the absence of toxicity (Mendoza et al. 1971).

In 1967 the executives and technicians of the Mexican company Sosa Texcoco rediscovered the fact that a species of alga grew naturally in the mineral and alkaline waters of a lake on the shores of which they had installed their plant for the production of sodium carbonate and sodium hydroxide. This was subsequently identified as Spirulina, a blue-green alga belonging to the Cyanophyceae group (Santillan, 1979).

They then constructed three pilot plants with continuing technical improvements, and carried out intensive cultivation with the assistance of the methodology supplied by the French Petroleum Institute which had originally developed a method for its cultivation (Bourges et al, 1971). At the present time the production capacity averages 25 tonnes/ha/year of dried biomass, representing 15 tonnes/ha/year of protein.

Spirulina, according to the descriptions found in the historical documents of Mexico, was consumed in the time of the Aztec empire and was used by them in the form of a cheese which the original inhabitants called "Tecuitatl" which in the Nahuatl language means "excrement of the stones". (Farrar, 1966, Durand-Chastell and David, 1977).

The algae which were consumed in Mexico up to about forty years ago were also cultivated and consumed in a rudimentary form by the natives of Central Africa, the place where these were encountered being Lake Chad whose waters, like those of the Texcoco lagoon, had the required characteristics of salinity, pH, light, temperature, etc. for its growth (Clement and Durand-Chastell, 1970).

From the time of this rediscovery of the Spirulina alga the Sosa Texcoco Company carried out the necessary studies in collaboration with the Instituto Nacional de la Nutrición de México. In its turn the United Nations Industrial Development Organization which showed a particular interest in the development of the project, was responsible for the carrying out of some important studies in national and foreign institutions. As a result of these the Consejo Nacional de Ciencias y Tecnología and the Instituto Nacional de la Nutrición organized scientific meetings in Paris and Mexico with the assistance of experts in the field of unicellular proteins (Santillan, 1979).

These various studies provided very interesting data on the aspects of cultivation, processing, nutritional qualities, chemical constitution and biological characteristics, making possible the installation of the present pilot plant at Sosa Texcoco and also the need for subsequent investigations.

The alga has a specific rate of growth which is higher than plants in agricultural cultivation, being more related to that of unicellular micro-organisms. This arises from the fact that Spirulina has an efficiency of absorption of solar energy of between 3 and 4.5% (Durand-Chastell and Santillan, 1975).

The present process for obtaining the dry alga in powder form involves the operations of preconcentration, vacuum filtration, fluidization, pasteurization, homogenization, and drying (Santillan, 1974).

From the nutritional point of view Spirulina represents a valuable source of protein because of its high content (65%) and its satisfactory composition in respect of the amino-acids present (Mendoza et al., 1971). It should be noted that the level of sulphur-containing amino-acids is satisfactory for metabolic utilization (Vermorel et al., 1973).

The PER has a value of 2.61 $\pm$ 0.15 and the NPU 56.6 $\pm$ 4.3 as compared with 2.97 and 61.5 respectively for standard casein (Bourges et al., 1971).

Its digestibility in rats is somewhat low according to Velmorel et al., (1975), but is improved by combining the alga with intact proteins (Kim, 1978). Spirulina does not, however, show the rigidity of the cellular walls of other algae (Cronshaw et al., 1958); and Northcote et al., 1958).

It has been found that the alga contains from 6 to 7% of total lipids, 83% of which consists of fatty acids (Hudson and Karis, 1974). Its carbohydrate content is from 13 to 17%, with a low energy value.

About ten vitamins have been identified together with substantial quantities of calcium, phosphorus, potassium and other trace elements which have an important function in nutrition (TNO, 1970).

From the point of view of toxicological analysis it has been found, from the determination of some elements, to comply with the recommendations which organizations such as the PAG (1974a) have suggested for non-conventional proteins so as to guarantee their safety in terms of human consumption.

The content of nucleic acids in the alga varies from 4.2 to 4.5%. The PAG (1975) suggests 2 g of nucleic acid per day as the maximum limit which unicellular proteins should provide in the adult diet. From this point of view it can be established that 46 grams of Spirulina constitute the upper limit for daily adult consumption (Bourges et al, 1971).

Tulliez et al., (1975) have also found that Spirulina alga contains from 0.1 to 0.3% of hydrocarbons, of which n-heptadecane is the major constituent (65%).

Determinations of 3,4-benzpyrene have been carried out on Spirulina dried on cylinders or by spraying, and these give respectively 2.6 and 3.8 ppb, relatively low concentrations in relation to the levels of several food products (Bories and Tulliez, 1975). Other workers report smaller concentrations (Dainipponik and Chemicals, 1977).

As far as the presence of metals and non-metals are concerned estimations have been carried out on samples at different times. Boudene et al. (1976) found principally a contamination by arsenic of up to 8.5 ppm, in contrast with the values of 0.7, 0.9, 1.1 and 2.4 ppm detected in other

studies (Japan Food Research Laboratories, 1977; TNO, 1976; LANFI, 1976). Cadmium was detected in quantities of 0.05 to 0.1 ppm; mercury at 0.01 to 0.6 ppm, selenium at 0.4 ppm and cyanide at 0.2 to 1.4 ppm. Analyses have also been carried out on the pesticide residues indicating only traces of 1,2,3,4,5,6-Hexachlorocyclohexane (BHC) in the α, β, γ and δ forms (Japan Food Research Laboratories, 1975).

In other determinations organochlorine and organophosphorus compounds were only detected at the level of traces of DDT and its metabolites (D.G.S.V., 1978).

In regard to the microbiological analysis there is a common flora in the cultivation consisting of halophytic protozoa and bacteria which are free growing; occasionally bacteria are detected of the coliform group. In the same way pathogens such as Salmonella, Shigella and E. Coli are absent (David et al., 1970). This is understandable, since the culture medium is of the hypersaline type with a strongly alkaline pH which is inhospitable for the invasion and growth of organisms which can not survive at such a pH (González et al., 1976; Contreras et al., 1979). The installation of the pasteurization equipment in the last stages of the process has an important function where the final product is concerned. Some authors have shown that various substances extracted from Spirulina show an anti-bacterial activity (Jacquet, 1976).

Spirulina has been used satisfactorily as a source of protein for pigs (Fevrier, 1976, Robles et al., 1975) and poultry (Bezares et al., 1976; Blum and Calet, 1976) and chickens

(Blum and Calet, 1975). In the same way the tolerance by rats fed for a hundred days was good, no abnormal observations being made in regard to growth, consumption of food, physical appearance, behaviour or any histopathological changes in the liver, lungs, kidneys or other organs examined (Bourges et al., 1971).

Till and Willems (1971) added 10% of Spirulina to the diet of rats for 90 days and did not encounter any changes in the above parameters or at a haematological level, biochemical analysis of the serum, analysis of the urine or the histopathology of 22 organs.

Tulliez et al. (1975) administered a diet with 25% of Spirulina which contained 280 ppm of heptodecane to rats and observed the accumulation of the latter principally in the adipose tissue. Other diets with 52 ppm of hydrocarbons administered to pigs showed slight retention and were well excreted in the milk. In none of the cases was there any manifestation of acute or chronic toxicity.

In a similar series of tests Boudene et al. (1976), feeding rats for 18 months, encountered some accumulation of arsenic but no signs of toxicity as shown by the increased weight, consumption of food mortality or histopathology of the 18 organs examined.

As far as humans were concerned a study carried out on undernourished subjects with 15, 30 and 50% of the protein

ration obtained from Spirulina did not cause modifications in the nutritional balance of nitrogen, sodium and potassium. The uric acid in the urine did not vary, although it did increase slightly in the serum (9 mg/1,000) (Sautier and Tremolieres, 1976).

Continuing with this type of study, and with the object of supplementing the toxicological examination of the alga, a series of small and large scale tests were carried out on laboratory animals, and the results of these are described in the present report.

These tests included sub-acute toxicity, chronic toxicity with functional tests, a multigeneration study of reproduction and lactation, mutagenesis and teratogenesis.

In the study of sub-acute toxicity above the level of 10% used by Till and Willems (1971), levels of 20% and 30% were used with the object of detecting any possible toxicity at these higher percentages without any imbalance in the nutrition.

By means of the tests for chronic toxicity with functional tests it was proposed to study the effects of the large-scale administration of Spirulina on the haematology, functioning of the kidneys, biochemistry of the serum and the weight and histology of some organs.

With the object of detecting any consequences of feeding the alga to successive generations a multigeneration study was devised in the rat, extended over a period of approximately

two years and completed in the last generation by a conventional study of sub-acute toxicity.

Finally investigations were carried out into possible mutagenicity in the rat and mouse and also on teratogenicity in these species and in the hamster.

Some data on the determination of metals, non-metals and pesticides which were carried out with the alga, parallel to the investigations on animals, have already been included in this introduction and will not be detailed subsequently.

STUDY OF SUB-CHRONIC TOXICITY OF SPIRULINA IN THE RAT

SUMMARY

Weaned Wistar rats were fed with levels of 10, 20 and 30% of Spirulina for three months. Observations were carried out on behaviour and appearance and a record was kept of the increase in weight and the consumption of food. Subsequently haematological analyses and analysis of the urine were carried out. The animals were sacrificed and determinations of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase and alkaline phosphatase were carried out on the serum. The organs were examined macroscopically and were subsequently submitted to histopathological examinations. No changes were observed which could be attributed to the consumption of Spirulina.

EXPERIMENTAL PART

Materials

The Spirulina used in this study was provided by the Sosa Texcoco Company, belonging to a bulked production batch.

Animals

Male and female weaned Wistar rats were used; the breeding stock of these was donated by the Instituto Miles de Terapéutica Experimental.

Diet

The Spirulina was incorporated in the experimental diet at levels of 10, 20 and 30%, gradually replacing soya meal. Two controlled diets were used, one of which consisted of a normal diet as used in the laboratory whilst the other was based on soya (see appended table). These diets were used in all the experiments.

Experimental design and methodology

Each of the five diets was given to batches consisting of 10 males and 10 females. The animals were grouped five to a cage and kept in a room at a temperature of $22 \pm 1^{o}C$, with light-dark cycles of twelve hours. Food and water was given "ad libitum".

EXPERIMENTAL DIET WITH ADDED SPIRULINA

AS USED IN THE STUDIES

INGREDIENTS	PERCENTAGE COMPOSITION			
Spirulina	-	10.0	20.0	30.0
Fish meal	8.0	8.0	8.0	8.0
Soya meal	44.4	29.6	14.0	-
Milled wheat grain	33.5	35.8	37.8	39.1
Milled yellow maize grain	11.2	11.9	12.7	13.2
Alfafa meal	-	2.0	5.0	6.6
Maize oil	1.6	1.6	1.6	1.6
Vitamins and minerals	0.7	0.7	0.7	0.7
Lysine	0.6	0.4	0.2	-
Methionine	-	-	-	0.1
Monosodium phosphate	-	-	-	0.7

The increase in weight was recorded each week; the consumption of food was also recorded each week for the first four weeks and subsequently in the 11th and 12th weeks.

In the 12th week samples of blood were taken by ocular puncture and on these the haematological analyses were carried out, consisting of the determinations of haemoglobin, haematocrit, erythrocytes and the total and differential leucocyte counts. At the end of the 13th week the urine was collected in metabolic cages for carrying out the corresponding analyses which included appearance, pH, glucose, protein, occult blood, ketones and microscopic examination of the sediment.

Subsequently the animals were sacrificed by decapitation and the serum was taken for analysis of glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), alkaline phosphatase and total serum protein (TSP), and was also examined macroscopically. The organs were also examined macroscopically, and the following were weighed: heart, brain, lungs, kidney, liver, spleen, testicles, ovaries, seminar vesicles and the thymus, thyroid, suprarenal and pituitary glands.

These organs were also submitted to histopathological studies, together with the pancreas, trachaea, salivary, mammary and lachrymatory glands, uterus, prostate, epididymis, gastro-intestinal tract, urinary bladder, skeletal muscle, spinal medula, femoral nerve, sternum, aorta and skin.

The results were submitted to statistical treatment in order to identify their significance by using the Wilcoxon or "t" tests: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, according to the case.

RESULTS

General condition of the animals

The general condition and behaviour of the animals was similar in the control batch and in the treated batches.

In all cases the appearance of the faeces of the experimental animals was of a dark colour, but there was no other change in regard to form and appearance.

During the course of the experiment one animal in the ordinary control batch died.

Increase in weight

Although slightly lower the body weight of the experimental batches showed no significant difference with the control batch on soya, neither in the males nor in the females (Table 1).

Consumption of food and efficiency

Table 2 shows that the batches treated with Spirulina ingested slightly smaller amounts than the controls, without this however being significant. This difference in consumption is reflected in the slight difference in the increase in weight.

Efficiency, for its part, was also similar in the case of both the males and the females, calculated from the first to the fourth week.

TABLE 1. INCREASE IN WEIGHT OF RATS FED WITH SPIRULINA FOR A PERIOD OF 13 WEEKS

Diet	Body weight (g) at week:							
	0	2	4	6	8	10	12	13
				M A L E S				
Ordinary control	58	124	194	253	288	323	339	352
Soya control	59	124	193	251	285	319	334	346
10% Spirulina	57	122	192	248	282	317	331	344
20% Spirulina	58	122	190	246	281	317	331	340
30% Spirulina	57	121	188	244	280	316	330	340
				F E M A L E S				
Ordinary control	56	105	140	166	182	197	205	211
Soya control	56	104	138	164	179	194	201	206
10% Spirulina	55	104	138	163	178	193	199	204
20% Spirulina	57	102	135	162	175	190	198	203
30% Spirulina	56	100	135	162	176	192	199	202

TABLE 2. CONSUMPTION AND CONVERSION EFFICIENCY IN RATS FED WITH SPIRULINA FOR A PERIOD OF 13 WEEKS

Diet	Consumption of food (g/rat/day) during week:						Efficiency*
	1	2	3	4	11	12	1st - 4th week
				MALES			
Ordinary control	8.8	11.8	14.9	16.2	16.8	17.2	0.37
Soya control	8.8	11.7	14.6	15.9	16.6	16.9	0.37
10% Spirulina	8.7	11.7	14.5	15.6	16.3	16.5	0.38
20% Spirulina	8.2	11.0	14.1	15.2	15.9	16.0	0.39
30% Spirulina	8.2	10.9	13.9	15.0	15.8	16.1	0.39
				FEMALES			
Ordinary control	8.0	10.8	11.5	12.0	12.4	12.6	0.28
Soya control	8.0	10.8	11.3	11.8	12.1	12.3	0.28
10% Spirulina	7.8	10.6	11.1	11.5	11.9	12.0	0.29
20% Spirulina	7.8	10.5	10.9	11.4	11.6	11.8	0.27
30% Spirulina	7.6	10.4	10.6	11.0	11.4	11.6	0.28

* weight gain/food consumed

Haematological analyses

Evaluation of the haematological parameters did not reveal any statistical difference in the values between the controls or any of the levels of Spirulina, as can be seen from Table 3.

Analysis of the serum

Terminal analysis of the examples of serum, with the object of determining the GOT, GPT, AP and TSP, did not show any changes which could be attributed to the Spirulina, as can be seen from Table 4.

Analysis of the urine

Table 5 shows that the urine of the control and treated animals shows no change in any of the parameters analysed during the whole of the three months.

Weight of the organs

Terminal autopsy did not show any significant differences in the relative weight of the organs, with the exception of the seminal vesicles in animals treated with 20 and 30% Spirulina (Table 6).

Macroscopic and histopathological observations

Macroscopic observation of the organs did not show any changes due to the consumption of the Spirulina. Nor did the histopathological examinations show any anomalies, as can be seen from Table 7, apart from those which were found both in the groups fed with Spirulina and also in the soya control batch.

TABLE 3. HAEMATOLOGICAL PARAMETERS FOR RATS FED WITH SPIRULINA FOR A PERIOD OF 13 WEEKS

Diet	Haemoglobin (g/100 ml)	Haematocrit (%)	Erythrocytes (10^6/mm^3)	Leucocytes Total (10^6/mm^3)	Differential Lymph.	neut.	eos.	mono.
MALES								
Ordinary control	14.7	48.6	7.3	13.9	82.3	14.4	3.0	0.3
Soya control	14.6	49.9	7.0	13.7	81.9	15.8	2.1	0.2
10% Spirulina	14.8	49.7	7.9	14.4	81.5	15.1	3.1	0.3
20% Spirulina	13.9	50.1	7.2	14.1	82.2	15.0	2.6	0.2
30% Spirulina	14.2	48.7	7.6	13.6	82.5	14.0	3.3	0.2
FEMALES								
Ordinary control	14.6	50.2	7.0	13.8	84.3	13.1	2.4	0.2
Soya control	14.3	49.0	6.7	13.2	86.0	12.3	1.6	0.1
10% Spirulina	14.0	48.8	7.0	14.0	85.3	12.7	1.8	0.2
20% Spirulina	14.1	48.7	6.9	13.9	84.8	13.6	1.4	0.2
30% Spirulina	14.0	49.2	6.7	12.8	83.8	13.9	2.0	0.3

TABLE 4. ANALYSIS OF THE SERUM OF RATS FED WITH SPIRULINA FOR A PERIOD OF 13 WEEKS

Diet	MALES				FEMALES			
	GOT (RFU)	GPT (RFU)	AP (BLU)	TSP g/100 ml	GOT (RFU)	GPT (RFU)	AP (BLU)	TSP g/100 ml
Ordinary control	158	32.2	5.3	6.3	147	25.3	5.0	6.6
Soya control	162	29.4	6.1	6.2	158	30.2	3.9	6.9
10% Spirulina	165	31.7	5.4	6.5	143	27.4	5.2	7.2
20% Spirulina	171	28.2	5.9	6.4	168	31.1	3.6	6.7
30% Spirulina	159	33.6	5.8	6.0	148	28.6	5.1	7.1

GOT = Glutamic-oxalacetic transaminase
GPT = Glutamic-pyruvic transaminase
AP = Alkaline phosphatase
TSP = Total serum protein
RFU = Reitman-Frankel unit
BLU = Bessey-Lowry unit

TABLE 5. ANALYSIS OF THE URINE OF RATS FED WITH SPIRULINA FOR A PERIOD OF 13 WEEKS

Diet	Appearance	pH	glucose	protein	occult blood	ketones	Analysis of sediment:					
							eryth.	leuc.	epith.	amorph.	cryst.	bact.
MALES												
Ordinary control	yellow	6	–	+	–	–	–	–	+	++	+	+
Soya control	yellow	6	–	+	–	–	–	–	+	+	+	+
10% Spirulina	yellow	5	–	+	–	–	–	–	+	++	+	+
20% Spirulina	yellow	6	–	+	–	–	–	–	+	++	+	+
30% Spirulina	yellow	5	–	+	–	–	–	–	+	+	+	+
FEMALES												
Ordinary control	yellow	5	+	+	–	–	–	–	+	++	–	+
Soya control	yellow	5	–	+	–	–	–	–	+	++	+	+
10% Spirulina	yellow	5	–	+	–	–	–	–	+	++	–	+
20% Spirulina	yellow	5	–	+	–	–	–	–	+	++	–	+
30% Spirulina	yellow	5	–	+	–	–	–	–	+	++	+	+

TABLE 6. RELATIVE WEIGHTS OF THE ORGANS OF RATS FED WITH SPIRULINA FOR A PERIOD OF 13 WEEKS

Diet	Body weight	Heart	Brain	Lungs	Kidney	Liver	Spleen	Testicles	Ovaries	Seminal vesicles	Thymus	Thyroids	Adrenals	Pituitary
						MALES								
Ordinary control	352	0.411	0.52	0.36	0.76	3.52	0.171	0.82	-	0.314	0.100	0.0070	0.0187	0.0033
Soya control	346	0.420	0.48	0.38	0.79	3.41	0.182	0.90	-	0.323	0.105	0.0071	0.0203	0.0037
10% Spirulina	344	0.408	0.50	0.41	0.81	3.49	0.193	0.86	-	0.364	0.096	0.0052	0.0186	0.0029
20% Spirulina	340	0.407	0.49	0.40	0.77	3.32	0.194	0.88	-	0.394*	0.102	0.0067	0.0210	0.0041
30% Spirulina	335	0.416	0.46	0.39	0.83	3.50	0.175	0.84	-	0.422*	0.090	0.0072	0.0168	0.0032
						FEMALES								
Ordinary control	211	0.423	0.80	0.51	0.72	3.37	0.194	-	0.025	-	0.129	0.0079	0.0223	0.0042
Soya control	206	0.437	0.71	0.42	0.83	3.20	0.171	-	0.028	-	0.136	0.0073	0.0251	0.0033
10% Spirulina	204	0.412	0.81	0.45	0.77	3.27	0.260**	-	0.031	-	0.128	0.0064	0.0218	0.0038
20% Spirulina	203	0.431	0.73	0.46	0.74	3.13	0.207*	-	0.030	-	0.129	0.0077	0.0274	0.0036
30% Spirulina	198	0.420	0.71	0.43	0.81	3.33	0.183	-	0.032	-	0.140	0.0075	0.0226	0.0040

TABLE 7. INCIDENCE OF HISTOPATHOLOGICAL OBSERVATIONS ON RATS FED WITH SPIRULINA OVER 13 WEEKS

Lesion	Diet*	Males					Females				
		A	B	C	D	E	A	B	C	D	E
	No. of rats examined	9	10	10	10	10	10	10	10	10	10
Kidneys											
Lymphoplasmocytaric infiltration with perivascular focus		1	2	1	0	1	1	0	1	1	0
Oedematous glomeruli		0	0	1	0	0	0	0	0	0	0
Lipoidic nephrosis		1	0	0	1	0	0	0	0	0	0
Mineral deposits in the pelvis		0	1	0	0	0	1	1	0	0	0
Unilateral hydronephrosis		1	2	1	2	2	1	0	1	1	0
Liver											
Infiltration by lymphocytes and neutrophilic leucocytes		0	0	0	1	0	0	0	0	0	0
Internal and cytoplasmic coleostasis		0	0	0	0	0	0	1	0	0	0
Enlargement of the basal portal membrane		0	0	0	0	0	0	0	0	0	1
Lungs											
Focal lymphoplasmocytaric infiltration		4	3	3	4	2	3	5	3	3	4
Focal infiltration of intralveolar polyblasts		0	0	1	0	0	0	0	0	0	0
Vascular congestion		0	0	0	0	0	0	0	1	1	0
Oedema		0	0	1	0	0	0	2	0	0	0
Heart											
Focal myocarditis		0	0	0	1	0	0	0	0	0	2
Bladder											
Lymphoplasmocytaric infiltration		0	0	0	0	0	1	1	0	0	1
Abundant polymorphonuclear leucocytes		1	0	0	0	0	0	0	1	0	0
Adrenal glands											
Hyperplasia of the glomerular tunic		1	0	0	0	0	0	0	0	0	1

* Diet A - ordinary control: B - soya control: C - 10% Spirulina: D - 20% Spirulina: E - 30% Spirulina

DISCUSSION

The results set out in the previous pages indicate that Spirulina fed to rats during a period of three months at levels of 10, 20 and 30% in the diet do not affect the parameters which were studied. The increase in weight of the males and females fed with the alga is slightly lower than that of the controls, but not significantly different.

This coincides with a slightly lower consumption of food in the case of experimental animals, but these show a similar conversion efficiency.

The haematological, serum and urine analyses show very similar values for the five batches.

Finally the very small significant differences in the weight of the organs and the results of the histopathological sections do not reflect any harmful effects of Spirulina.

STUDIES OF CHRONIC TOXICITY CARRIED OUT ON RATS FED WITH SPIRULINA FOR A PERIOD OF 21 MONTHS

SUMMARY

Wistar rats were fed over a period of 21 months with 10, 20 and 30% Spirulina. Two control batches received ordinary food and a diet based on soya respectively. The increase in weight was similar in the batches which received the Spirulina and the soya diets. In the same way there were similarities in the results of the haematological analyses and the functional tests and also in the terminal chemical analysis of the serum. The relative weights of the organs and the histopathological examination did not show any differences of pathological interest, whether in respect of lesions in the tissues or in the incidence of tumours. It is concluded that Spirulina does not produce any toxic effects in the parameters studied and under the experimental conditions.

EXPERIMENTAL PART

Materials

Spirulina from the Sosa Texcoco Company was used, obtained by the process previously described.

Animals

Wistar male and female rats were used, provided by the Instituto Miles de Terapéutica Experimental.

Diets

As in the other experiments the Spirulina was incorporated into the diet at levels of 10, 20 and 30% at the expense of the soya. Two control batches were used, one of the diets being based on soya whilst the other consisted of a commercial diet commonly used in the laboratory. The diets were prepared approximately every three or four weeks and were stored at the temperature of the room.

Procedure

Five batches, each containing twenty males and twenty females, were established at random, and these were given the diets indicated above.

Throughout the whole experiment they remained in rooms under controlled environmental conditions.

The animals were grouped five per cage, and had free access to food and water. Their weight was checked every month. Determinations of the haematological parameters were made at 48, 64 and 80 weeks, comprising haemoglobin, haematocrit, erythrocytes and total and differential leucocyte counts.

At the same time tests were carried out on renal function by measuring the elimination of phenol red, determination of the specific gravity and the glutamic-pyruvic transaminase in the urine, at the same intervals of time.

The surviving animals were sacrificed in the 84th week. Samples of the blood were taken for biochemical examination, and on these were carried out determinations of glucose, ureic nitrogen (BUN), glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), alkaline phosphatase (AP) and total serum protein (TSP).

Also carried out were macroscopic analyses of the organs and the weight of the heart, brain, lungs, kidneys, liver, spleen, testicles, ovaries, seminal vesicles and the thyroid, adrenal and pituitary glands.

Subsequently a histopathological examination was carried out on these organs, and also on the pancreas, trachaea, salivary glands, mammary glands, uterus, prostate, epididymis, gastro-intestinal tract, urinary bladder, skeletal muscle, spinal medula, femoral nerve, aorta and skin.

The results were subjected to statistical treatment to identify the significance by means of the Wilcoxon or "t" tests: *P < 0.05; **P < 0.01; ***P < 0.001, according to the case.

RESULTS

Increase in weight

Body weights were almost identical in all the batches of animals up to the fourth month. From then onwards the soya controls began to gain less than the ordinary controls, so that at the end of the 21 months there was a difference of 5.8% between the males and 7.6% between the females (Table 1). There was no difference in those treated with the soya control and in the two sexes, although with 30% Spirulina the weights were consistently lower.

Haematological analyses

The haematological parameters recorded during the 48 weeks were not affected by the consumption of Spirulina at the various levels (Table 2). In the 64th week a significant difference was noticed in the haematocrit values with 10% Spirulina (Table 3). Furthermore it can be seen from Table 4 that there are significant differences in the concentration of haemoglobin and the number of erythrocytes in the case of the males fed with 20 and 10% of the alga respectively.

Functional tests on the kidneys

Table 5 shows that the ingestion of Spirulina does not affect the excretion of phenol red, the specific gravity or the glutamic-oxalacetic transaminase activity in the urine. There is only a significant difference in the specific gravity in the

TABLE 1. FINAL MEAN BODY WEIGHT OF SURVIVING RATS FED WITH SPIRULINA FOR A PERIOD OF 21 MONTHS

Diet	No. of surviving rats	Mean final weight	
		Weight (g)	Difference from soya control (%)
	MALES		
Ordinary control	13	653	+ 5.8
Soya control	12	617	
10% Spirulina	12	613	- 0.6
20% Spirulina	11	611	- 1.0
30% Spirulina	12	631	+ 2.3
	FEMALES		
Ordinary control	10	412	+ 7.6
Soya control	9	383	
10% Spirulina	12	373	- 2.6
20% Spirulina	11	376	- 1.8
30% Spirulina	12	391	+ 2.1

TABLE 2. HAEMATOLOGICAL PARAMETERS FOR RATS FED WITH SPIRULINA FOR A PERIOD OF 48 WEEKS

Diet	Haemo-globin (g/100 ml)	Haemato-crit (%)	Erythro-cytes (10^6/mm^3)	Leucocytes				
				Total (10^6/mm^3)	Differential			
					Lymph.	neut.	eos.	mono.
MALES								
Ordinary control	14.5	49.2	7.7	13.7	81.2	15.8	2.8	0.2
Soya control	14.8	48.7	8.1	14.2	82.5	14.6	2.9	0.0
10% Spirulina	15.1	49.5	7.8	13.9	82.4	13.9	3.4	0.3
20% Spirulina	14.7	50.0	8.2	14.0	82.4	14.1	3.3	0.2
30% Spirulina	14.9	50.1	7.9	14.1	85.1	11.3	3.4	0.2
FEMALES								
Ordinary control	15.0	48.3	7.0	13.2	82.7	14.6	2.6	0.1
Soya control	14.6	47.8	7.4	13.5	83.1	13.7	2.9	0.3
10% Spirulina	14.8	48.2	7.2	12.8	84.1	12.4	3.3	0.2
20% Spirulina	14.6	49.2	7.5	12.7	83.1	13.2	3.5	0.2
30% Spirulina	14.7	48.6	7.3	13.4	86.2	10.7	3.0	0.1

TABLE 3. HAEMATOLOGICAL PARAMETERS FOR RATS FED WITH SPIRULINA FOR A PERIOD OF 64 WEEKS

Diet	Haemo-globin (g/100 ml)	Haemato-crit (%)	Erythro-cytes (10^6/mm^3)	Leucocytes				
				Total (10^6/mm^3)	Differential			
					Lymph.	neut.	eos.	mono.
MALES								
Ordinary control	14.8	49.6	7.9	13.8	79.7	16.6	3.5	0.2
Soya control	14.6	49.8	8.4	14.8	80.7	16.0	2.9	0.4
10% Spirulina	14.9	50.9*	7.9	14.6	80.9	15.4	3.3	0.4
20% Spirulina	14.6	50.3	8.3	13.8	78.2	17.8	3.7	0.3
30% Spirulina	14.7	49.7	8.1	14.5	79.9	17.2	2.8	0.1
FEMALES								
Ordinary control	14.6	47.5	7.3	13.2	78.8	17.5	3.4	0.3
Soya control	14.8	47.2	7.8	14.0	80.9	15.8	3.0	0.3
10% Spirulina	14.8	48.8	7.6	13.9	82.3	14.7	2.6	0.4
20% Spirulina	14.5	47.4	7.9	13.3	80.5	15.2	4.1	0.2
30% Spirulina	15.1	47.3	7.7	13.8	80.7	13.9	3.2	0.2

TABLE 4. HAEMATOLOGICAL PARAMETERS FOR RATS FED WITH SPIRULINA FOR A PERIOD OF 80 WEEKS

Diet	Haemo-globin (g/100 ml)	Haemato-crit (%)	Erythro-cytes (10^6/mm^3)	Leucocytes				
				Total (10^6/mm^3)	Lymph.	neut.	eos.	mono.
			MALES					
Ordinary control	14.6	49.7	8.0	16.5	78.5	18.8	3.0	0.2
Soya control	15.0	50.3	8.2	15.2	76.5	19.6	3.5	0.4
10% Spirulina	14.8	50.0	9.1*	14.6	74.4	21.3	4.2	0.1
20% Spirulina	14.2*	48.8	8.3	16.3	79.4	18.6	1.9	0.1
30% Spirulina	14.7	50.2	8.4	14.8	75.3	22.5	2.0	0.2
			FEMALES					
Ordinary control	15.3	47.6	7.0	13.1	78.5	18.6	2.6	0.3
Soya control	15.1	48.5	7.6	12.8	76.7	20.1	3.1	0.1
10% Spirulina	14.8	47.2	7.3	13.4	77.3	20.5	2.0	0.2
20% Spirulina	14.9	47.0	7.5	11.7	78.4	19.7	1.7	0.2
30% Spirulina	14.7	48.4	7.2	12.5	78.8	19.4	1.7	0.1

ordinary control females ($P < 0.01$) as compared with the soya controls.

Table 6 shows similar results, although significant differences can be seen in the specific gravity of the urine of males fed with 20% Spirulina and in the glutamic-oxalacetic transminase in females belonging to the ordinary control group.

Similarly differences are found in the same parameters between females treated over a period of 80 weeks with 10 and 20% of the alga and in the ordinary control respectively (Table 7).

Analysis of the serum

Biochemical determinations on the serum of samples taken at 84 weeks do not show any significant differences between the groups fed with Spirulina and the soya controls (Table 8). There is, however, a difference in the ureic nitrogen of the males in the ordinary control and also in the alkaline phosphatase of the females of the same group.

Weight of organs

The relative weights of the organs are shown in table 9. In the males there is a significant difference in the relative weights of the hearts with 10% Spirulina, of the lungs with 20%, and of the kidneys in the animals of the ordinary control group. In the females a difference is found between the relative weight of the adrenals as compared with the ordinary controls.

TABLE 5. RESULTS OF FUNCTIONAL TESTS ON THE KIDNEYS OF RATS FED WITH SPIRULINA FOR A PERIOD OF 48 WEEKS

Diet	Excretion of phenol red (% in one hour)	Specific gravity	GOT (RFU)
		MALES	
Ordinary control	48.2	1.0513	10.1
Soya control	46.9	1.0636	9.6
10% Spirulina	50.5	1.0549	10.4
20% Spirulina	47.6	1.0654	8.8
30% Spirulina	49.3	1.0627	9.5
		FEMALES	
Ordinary control	67.7	1.0783**	10.4
Soya control	64.5	1.0600	10.7
10% Spirulina	68.1	1.0642	9.8
20% Spirulina	66.8	1.0562	10.2
30% Spirulina	64.2	1.0691	11.1

TABLE 6. RESULTS OF FUNCTIONAL TESTS ON THE KIDNEYS OF RATS FED WITH SPIRULINA FOR A PERIOD OF 64 WEEKS

Diet	Excretion of phenol red (% in one hour)	Specific gravity	GOT (RFU)
		MALES	
Ordinary control	50.7	1.0661	11.4
Soya control	48.4	1.0546	11.6
10% Spirulina	50.3	1.0678	10.7
20% Spirulina	47.6	1.0750**	9.3
30% Spirulina	51.1	1.0523	10.1
		FEMALES	
Ordinary control	66.5	1.0543	8.1*
Soya control	65.2	1.0561	10.2
10% Spirulina	67.8	1.0629	10.7
20% Spirulina	63.4	1.0546	11.6
30% Spirulina	63.6	1.0692	9.8

TABLE 7. RESULTS OF FUNCTIONAL TESTS ON THE KIDNEYS OF RATS FED WITH SPIRULINA FOR A PERIOD OF 80 WEEKS

Diet	Excretion of phenol red (% in one hour)	Specific gravity	GOT (RFU)
	MALES		
Ordinary control	47.8	1.0622	12.6
Soya control	49.5	1.0689	10.3
10% Spirulina	49.5	1.0544	10.0
20% Spirulina	51.7	1.0560	11.2
30% Spirulina	47.3	1.0595	9.3
	FEMALES		
Ordinary control	59.8	1.0646	14.6**
Soya control	64.6	1.0630	9.9
10% Spirulina	66.3	1.0722*	9.6
20% Spirulina	58.2	1.0754*	10.1
30% Spirulina	63.7	1.0586	10.7

TABLE 8. ANALYSIS OF THE SERUM OF RATS FED WITH SPIRULINA FOR A PERIOD OF 84 WEEKS

Diet	MALES						FEMALES					
	Glucose (mg/100 ml)	BUN (mg/100 ml)	GOT (RFU)	GPT (RFU)	AP (BLU)	TSP g/100 ml	Glucose (mg/100 ml)	BUN (mg/100 ml)	GOT (RFU)	GPT (RFU)	AP (BLU)	TSP g/100 ml
Ordinary control	71	9.3*	141	30.8	5.8	6.2	68	10.8	132	25.7	3.2*	6.8
Soya control	68	16.3	150	29.7	5.0	6.5	66	12.6	141	27.3	5.1	6.0
10% Spirulina	72	14.8	147	25.5	3.8	5.9	71	11.5	145	24.6	4.2	4.9
20% Spirulina	65	13.6	145	28.3	4.5	6.4	65	12.2	148	28.0	3.9	4.6
30% Spirulina	68	14.1	153	31.6	3.6	6.7	67	10.7	142	26.4	4.4	4.1

BUN = Bilirubin ureic nitrogen
GOT = Glutamic-oxalacetic transaminase
GPT = Glutamic-pyruvic transaminase
AP = Alkaline phosphate
TSP = Total serum protein
RFU = Reitman-Frankel units
BLU = Bessey-Lowry units

Macroscopic and histopathological observations

The survival of the animals fed with Spirulina was slightly higher than the animals in the soya control, and for this reason the Peto treatment (1974) was not applied to the results.

In the study carried out by Boudene et al. (1976) changes were encountered in the mammary glands, uterus, skin and peritoneum.

Macroscopic examination of the organs did not show any pathological changes which could be attributed to the Spirulina, being those which could be expected in animals of an advanced age. Furthermore no histological differences were encountered in the groups in respect of non-tumoral or tumoral lesions (Tables 10 and 11). All the tumours were of the types commonly found in this race of rats.

TABLE 9. RELATIVE WEIGHTS OF THE ORGANS OF RATS FED ON SPIRULINA FOR A PERIOD OF 84 WEEKS

Diet	Heart	Brain	Lungs	Kidneys	Liver	Spleen	Testicles	Ovaries	Seminal vesicles	Thyroids	Adrenals	Pituitaries
						MALES						
Ordinary control	0.398	0.46	0.37	0.98*	3.44	0.168	0.75	-	0.309	0.0061	0.0179	0.0030
Soya control	0.407	0.51	0.38	0.77	3.37	0.165	0.80	-	0.318	0.0074	0.0165	0.0034
10% Spirulina	0.431*	0.48	0.35	0.82	3.49	0.166	0.78	-	0.349	0.0065	0.0174	0.0028
20% Spirulina	0.388	0.47	0.56*	0.76	3.33	0.170	0.76	-	0.315	0.0068	0.0178	0.0038
30% Spirulina	0.392	0.50	0.35	0.73	3.22	0.175	0.69	-	0.326	0.0071	0.0139	0.0029
						FEMALES						
Ordinary control	0.382	0.74	0.41	0.70	3.39	0.180	-	0.026	-	0.0078	0.0250**	0.0047
Soya control	0.410	0.68	0.44	0.68	3.41	0.177	-	0.030	-	0.0082	0.0198	0.0040
10% Spirulina	0.393	0.71	0.42	0.77	3.40	0.192*	-	0.024	-	0.0074	0.0223	0.0042
20% Spirulina	0.387	0.70	0.48	0.74	3.37	0.179	-	0.031	-	0.0080	0.0230	0.0051
30% Spirulina	0.412	0.67	0.46	0.73	3.32	0.188	-	0.033	-	0.0081	0.0184	0.0048

TABLE 10. INCIDENCE OF HISTOPATHOLOGICAL OBSERVATIONS ON RATS FED WITH SPIRULINA OVER 21 MONTHS

Lesion	Diet*	Males					Females				
		A	B	C	D	E	A	B	C	D	E
	No. of rats examined	13	12	12	11	12	10	9	12	11	12
Kidneys											
Moderate to severe chronic infirmity		8	7	6	7	8	5	7	6	7	6
Calcareous deposits		1	1	0	0	0	0	0	1	2	1
Arterial hyalinosis		0	0	1	0	1	0	1	0	0	0
Enlargement of the basal glomerular capillaries		0	0	0	0	0	0	1	0	0	0
Bilateral hydronephrosis		1	1	0	1	0	1	0	0	0	0
Liver											
Infiltration by lymphocytes and neutrophilic leucocytes		1	1	0	0	0	0	0	1	0	1
Cellular vacualization		1	1	2	2	1	0	2	1	0	1
Hyalinization of the arteriolar walls		0	1	0	1	1	1	1	0	0	1
Necrosis		0	0	1	0	1	0	1	0	1	1
Lungs											
Chronic respiratory infirmity		3	3	2	4	4	3	5	4	5	4
Oedema		1	0	1	1	0	0	1	0	1	0
Calcification of the arteries		0	1	1	0	0	0	0	0	0	0
Pancreas											
Periarteritis		0	0	1	0	0	1	0	0	0	1
Uterus											
Inflammatory reaction		0	0	0	0	0	2	1	0	0	0
Testicles											
Atrophy		1	0	0	0	0	0	0	0	0	0
Prostate											
Inflammatory reaction		2	1	1	0	1	0	0	0	0	0

TABLE 10. (continued)

Lesion	Diet*	Males					Females				
		A	B	C	D	E	A	B	C	D	E
	No. of rats examined	13	12	12	11	12	10	9	12	11	12
Bladder											
Lymphoplasmocytaric infiltration		0	0	0	1	0	0	1	0	0	0
Abundant polymorphonuclear leucocytes		0	1	0	1	0	0	0	0	0	0
Ovaries											
Partial atrophy		0	0	0	0	0	0	0	1	0	0
Adrenal glands											
Haemorrhagic cyst of cortex		0	0	1	0	1	0	0	0	0	0
Muscles											
Granular hyaline inclusions		0	0	1	1	0	0	0	0	0	0

* Diet A - ordinary control: B - soya control: C - 10% Spirulina: D - 20% Spirulina: E - 30% Spirulina

TABLE 11. INCIDENCE AND TYPE OF TUMOURS IN RATS FED WITH SPIRULINA OVER 84 WEEKS

Site and type of tumour	Diet*	Number of tumours: Males A	B	C	D	E	Females A	B	C	D	E
	No. of rats examined	13	12	12	11	12	10	9	12	11	12
	No. of rats with tumours	10	9	7	8	9	9	7	9	8	8
Lungs											
Pulmonary adenoma		1	0	1	0	0	0	0	0	0	1
Lymphoreticular tumour		3	4	2	1	3	2	1	2	3	1
Mammary glands											
Fibroadenoma		0	1	0	0	0	4	3	4	2	3
Fibroma		0	1	0	0	0	0	1	0	0	1
Adenocarcinoma		0	0	0	0	0	1	0	1	1	0
Skin											
Dermatofibroma		1	0	1	0	0	0	1	0	0	0
Keratoacanthoma		0	0	0	1	1	0	0	0	1	0
Thyroid glands											
Folicular carcinoma		0	1	0	0	0	0	0	0	0	0
Trabecular adenoma		2	2	2	2	3	1	2	1	1	1
Pituitary gland											
Adenoma of the anterior lobule		2	1	1	1	0	2	1	0	0	0
Adenocarcinoma		0	0	1	0	0	1	0	1	0	0
Small adenoma		0	0	0	0	1	1	0	0	1	0
Suprarenal glands											
Cortical adenoma		3	1	1	2	2	0	1	0	1	0
Chromaffinoma		2	1	0	2	1	0	0	0	0	1
Subcutaneous tissues											
Fibroma		0	0	1	0	0	0	0	0	0	0
Fibrosarcoma		0	1	1	0	0	1	0	0	1	0

TABLE 11. (continued)

Large intestine										
Lymphosarcoma	1	1	0	1	0	0	0	0	1	0
Small intestine										
Lymphosarcoma	1	0	1	0	0	1	0	0	0	1
Pancreas										
Adenoma	0	0	0	1	2	0	0	0	1	0
Ovaries										
Sarcoma	0	0	0	0	0	1	1	1	0	0
Uterus										
Fibroma	0	0	0	0	0	0	1	1	0	1
Total number of tumours	16	14	12	11	13	15	12	11	13	10

* Diet A - ordinary control: B - soya control: C - 10% Spirulina
D - 20% Spirulina: E - 30% Spirulina

DISCUSSION

The results described above show that Spirulina, at levels of 10, 20 and 30% in the diet of Wistar rats during a period of 21 months, does not produce any harmful effects in the parameters studied.

The growth of the animals in the treated groups followed a curve similar to the soya control, and this agrees with the results of Boudene et al. (1976) in the sense that there were no changes in the rate of growth. This author points out also that the growth of the males was slightly faster than the controls in those batches which were using casein as a source of protein.

No significant differences were found in the haematological parameters which could be attributed to the ingestion of the alga, since those which were found did not have any relationship to the level of Spirulina fed and were not subsequently encountered.

The functional tests carried out in the course of various weeks did not indicate any harmful effects, and in this way agreed with the results of the study carried out on the F_{3b} generation of the multigeneration tests on reproduction and lactation. The frequency with which these determinations were carried out were considered adequate for the period of 21 months which the experiment lasted, since data were also available on the study of sub-acute toxicity and in which the same concentrations of the alga

were used.

The survival of the animals treated with the alga were equal or slightly higher than the soya controls, so that this excludes the possibility that the Spirulina contains any toxic agent which, during a prolonged period, would interfere with the normal physiological or biochemical processes. These results were reflected in the biochemical analysis of the serum carried out at the end of the experiment, in which there was seen only a change in the ureic nitrogen and the alkaline phosphatase in the ordinary control batch.

The post-mortem results, including the relative weight of the organs, and the histopathological study, do not suggest any harmful effects from Spirulina.

MULTIGENERATION STUDY ON REPRODUCTION AND LACTATION IN RATS FED WITH SPIRULINA

SUMMARY

Spirulina was fed at levels of 10, 20 and 30% to batches of Wistar rats in a multigeneration study (three generations over approximately two years). Two control batches received a commercial diet and a diet based on soya respectively. The fertility, gestation, viability and lactation indices were recorded. A study of sub-acute toxicity was carried out on the F_{3b} generation, covering the general condition, increase in weight, consumption and conversion efficiency, haematology, serum and urine analysis, weight and histopathological examination of the organs. This multigeneration study revealed no effects on fertility, size of the litters or mortality in the period set out for testing. Similarly the tests on sub-acute toxicity, including the histopathological examinations, showed no anomalies relating to the treatment. In conclusion, therefore, Spirulina does not cause any detectable effects on the parameters investigated.

EXPERIMENTAL PART

Materials

For this study Spirulina was used from the Sosa Texcoco Company, corresponding to a bulked production batch.

Animals

Wistar male and female weaned rats were used, donated by the Instituto Miles de Terapéutica, and these were placed in groups of 5 animals. The room in which this study was carried out had a temperature of 22 $\pm$ 1°C, controlled humidity, and cycles of light and dark of 12 hours (8.00 - 20.00 hours).

Diet

Five different diets were used, three of which were prepared with 10, 20 and 30% Spirulina, the two remaining diets forming the controls. One of the controls consisted of an ordinary diet, commonly used in the laboratory, and the other was prepared on a basis of soya meal.

The Spirulina was added mainly at the expense of the soya.

Procedure

The rats were distributed at random into five batches of twenty females and ten males each, thus forming the F_o

generation. The diet and water were provided "ad libitum" during the whole of the study. At the end of the 12th and 20th weeks the males and females were mated over a period of three weeks, and at the end of this period the females were placed in individual cages for the birth of the F_{1a} and F_{1b} generations (Fig. 1). The live and dead young were counted, and the litter was weighed at days 4 and 21, the period during which weaning was carried out. This makes it possible to calculate the indices of fertility (F.I.), gestation (G.I.), viability (V.I.) and lactation (L.I.), which represent respectively the percentages of matings giving rise to gestating females, the percentage of gestating females giving rise to the birth of young, the percentage of young who survived for four days and the percentage of living young at 4 days which survived to the 21st days of the period of lactation.

The F_{1a} generation was sacrificed and autopsies were carried out. From the F_{1b} generation ten males and twenty females were selected at random from each batch, and these were subjected to the same treatment as their parents. At three months of age they were mated successively on two opportunities to obtain the F_{2a} and F_{2b} generations. This procedure was then followed with the F_2 generation in the same way as with the F_1 generation, and the F_{3a} and F_{3b} generations which resulted were sacrificed and autopsies carried out on weaning and at fourteen weeks respectively.

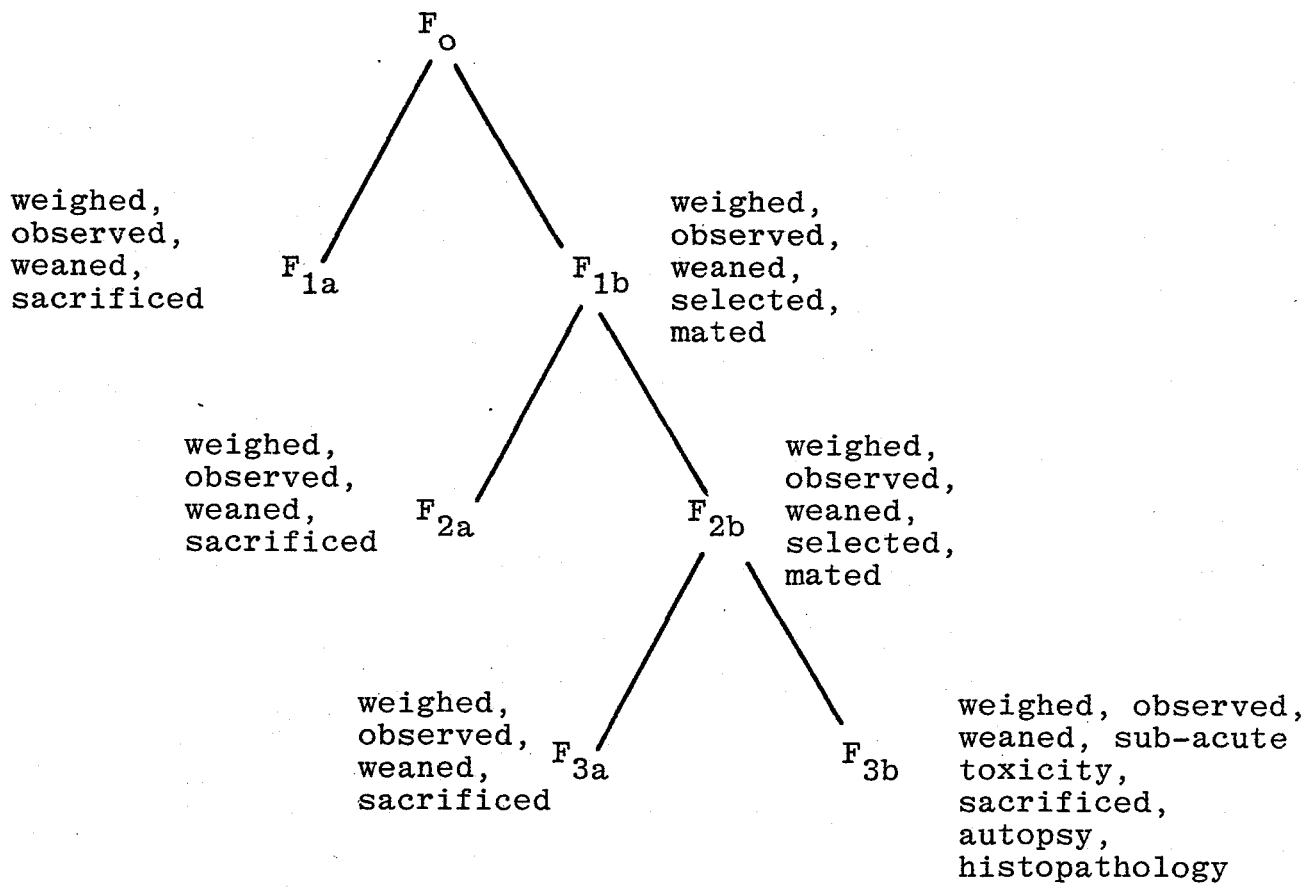


Fig. 1. SCHEME FOR THE MULTIGENERATION STUDY OF REPRODUCTION AND LACTATION. After Fitzhugh, O.G. (1968) : Reproduction tests. From "Modern Trends in Toxology, I" Eds. E. Boyland and G. Goulding, Butterworths, London.

Before the sacrifice of the F_{3b} generation ten males and ten females were selected at random to carry out a conventional study of sub-acute toxicity. For this the animals were weighed fortnightly and the consumption of food was measured during the first four weeks and also in the 11th and 12th weeks. At the end of the 13th week samples of blood were taken for a haematological examination which included the determination of haemoglobin, haemocrit, erythrocytes and a total and differential leucocyte count. Also carried out were analyses of the urine including appearance, pH, glucose, proteins, occult blood, ketones and a macroscopic study of the sediment. Functional tests on the kidneys were made by measuring the phenol red in the urine, the density and the glutamic-oxalacetic transaminase activity. At the end of the study the animals were sacrificed by decapitation and samples of blood were taken for analysis and also for analysis of the glutamic-pyruvic transaminase, glutamic-oxalacetic transaminase, alkaline phosphatase and total serum protein. Autopsies were carried out, and the organs were examined macroscopically and weighed: heart, brain, lungs, kidney, liver, spleen, testicles, ovaries, seminal vesicles and the thymus, thyroid, adrenal and pituitary glands.

Subsequently a histopathological examination was carried out on these organs and also on the pancreas, trachaea, salivary, mammary and lachrymatory glands, uterus, prostate, epididymis, gastro-intestinal tract, urinary bladder, skeletal muscles, spinal medula, femoral nerve, sternum, aorta and skin.

RESULTS

Tables 1 to 6 show the results of study of reproduction and lactation in which 100 litters were obtained from the ordinary controls, 114 from the soya controls, 97 from the 10% Spirulina, 95 for the 20% Spirulina and 99 from the 30% Spirulina.

In Table 1, relating to the first mating of the F_o generation, it will be seen that the fertility index is similar in the control batches and in those treated with Spirulina at different levels. It will be noted that almost all the matings gave rise to pregnancies.

The index of gestation also shows that the pregnant animals gave rise to terminations in percentages ranging from 94% to 100% in the various batches, but no negative effects of the alga in regard to this parameter were observed.

As far as the litters in themselves were concerned no modifications were observed, either in the viability index or in the lactation index. These all exceeded values of 90%.

The mean weight of the litters recorded on days 4 and 21 did not show any experimental changes in relation to the controls.

The results corresponding to the second mating of this generation are shown in Table 2. Although different indices are found, being reduced in relation to the results of the first matings, the figures are similar when compared with the soya controls.

In the same way the mean weight of the litters produced by mothers treated with Spirulina do not differ materially from the controls.

Table 3 shows that the indices are not affected as compared with the respective soya controls.

In the case of the lactation index the values fluctuate between 77 and 87%, that is to say they are slightly reduced when compared with the corresponding figures from the first mating.

There is no variation between the different groups for each period of the growth of the litters during weaning.

Table 4 shows the results of the second mating of the F_{1b} generation, and this indicates that the indices are not found to be modified by feeding with Spirulina. The weight of the litters also shows no normal effect of the alga.

The data for the F_{2b} generation is shown in Tables 5 and 6. In the table referring to the first mating it will be seen that the four indices being considered show no variations between the control and those treated with Spirulina, indicating that no changes are being produced in the reproductive functions.

As in the previous cases the mean weight of the litters is not affected, either at 4 days or on the day of weaning.

TABLE 1. SPIRULINA AND THE REPRODUCTION AND LACTATION OF THE F_0 GENERATION OF RATS: FIRST MATING

Treatment	No. of matings	No. of pregnancies	No. of births	Mean litters/female: Born	4 days	weaned	Mean weight of litters at: day 4	day 21	F.I.	G.I.	V.I.	L.I.
Ordinary control	20	18	18	8.5	8.5	8.0	8.2	40.0	90	100	100	94
Soya control	21	20	20	8.0	8.0	7.5	8.0	41.3	95	100	100	94
10% Spirulina	20	18	17	8.3	8.0	8.0	8.1	40.9	90	94	96	100
20% Spirulina	18	16	15	7.9	7.8	7.0	8.3	41.0	89	94	99	90
30% Spirulina	20	19	18	8.8	8.3	8.1	7.8	41.4	95	95	94	94

F.I. = Fertility index
G.I. = Gestation index
V.I. = Viability index
L.I. = Lactation index

TABLE 2. SPIRULINA AND THE REPRODUCTION AND LACTATION OF THE F_0 GENERATION OF RATS: SECOND MATING

Treatment	No. of matings	No. of pregnancies	No. of births	Mean litters/female: Born	4 days	weaned	Mean weight of litters at: day 4	day 21	F.I.	G.I.	V.I.	L.I.
Ordinary control	20	20	18	9.6	8.4	7.4	8.3	41.3	100	90	87	88
Soya control	20	20	19	8.6	7.2	6.3	8.0	39.8	100	95	84	87
10% Spirulina	20	19	19	9.8	8.8	8.1	8.4	40.7	95	100	90	92
20% Spirulina	20	18	18	8.3	7.8	7.0	7.8	40.4	90	100	94	90
30% Spirulina	20	18	16	8.7	8.0	7.4	8.5	41.0	90	89	92	92

F.I. = Fertility index

G.I. = Gestation index

V.I. = Viability index

L.I. = Lactation index

TABLE 3. SPIRULINA AND THE REPRODUCTION AND LACTATION OF THE F_{1b} GENERATION OF RATS: FIRST MATING

Treatment	No. of matings	No. of pregnancies	No. of births	Mean litters/female: Born	4 days	weaned	Mean weight of litters at: day 4	day 21	F.I.	G.I.	V.I.	L.I.
Ordinary control	20	18	17	9.1	8.0	6.4	8.4	41.3	90	94	88	80
Soya control	20	17	17	9.3	8.3	6.7	8.5	40.8	85	100	89	81
10% Spirulina	19	16	15	8.8	7.5	5.9	8.2	38.6	84	94	85	77
20% Spirulina	18	15	15	8.5	7.3	6.0	8.3	40.0	83	100	86	82
30% Spirulina	19	18	17	8.7	7.8	6.8	7.9	40.5	95	94	90	87

F.I. = Fertility index
G.I. = Gestation index
V.I. = Viability index
L.I. = Lactation index

TABLE 4. SPIRULINA AND THE REPRODUCTION AND LACTATION OF THE F_{1b} GENERATION OF RATS: SECOND MATING

Treatment	No. of matings	No. of pregnancies	No. of births	Mean litters/female: Born	4 days	weaned	Mean weight of litters at: day 4	day 21	F.I.	G.I.	V.I.	L.I.
Ordinary control	19	16	15	8.8	7.3	6.2	8.6	40.8	84	94	83	85
Soya control	20	18	16	8.4	7.3	5.5	8.6	41.4	90	89	82	87
10% Spirulina	19	17	15	9.2	7.6	6.6	8.2	39.1	89	88	83	87
20% Spirulina	18	15	14	9.0	8.0	6.2	8.4	41.2	83	93	89	85
30% Spirulina	18	18	17	8.7	8.1	7.5	8.1	40.3	100	94	93	92

F.I. = Fertility index
G.I. = Gestation index
V.I. = Viability index
L.I. = Lactation index

TABLE 5. SPIRULINA AND THE REPRODUCTION AND LACTATION OF THE F_{2b} GENERATION OF RATS: FIRST MATING

Treatment	No. of matings	No. of pregnancies	No. of births	Mean litters/female: Born	4 days	weaned	Mean weight of litters at: day 4	day 21	F.I.	G.I.	V.I.	L.I.
Ordinary control	20	18	16	8.9	8.4	7.8	8.1	40.0	90	89	94	93
Soya control	18	17	16	9.0	8.4	7.6	8.2	40.6	94	94	93	90
10% Spirulina	20	18	16	8.8	8.2	7.3	8.4	41.1	90	89	93	89
20% Spirulina	19	18	17	9.3	8.6	7.7	8.0	41.4	95	94	92	89
30% Spirulina	19	18	16	9.5	8.9	8.2	8.5	40.9	95	89	94	92

F.I. = Fertility index
G.I. = Gestation index
V.I. = Viability index
L.I. = Lactation index

TABLE 6. SPIRULINA AND THE REPRODUCTION AND LACTATION OF THE F_{2b} GENERATION OF RATS: SECOND MATING

Treatment	No. of matings	No. of pregnancies	No. of births	Mean litters/female: Born	4 days	weaned	Mean weight of litters at: day 4	day 21	F.I.	G.I.	V.I.	L.I.
Ordinary control	20	18	16	9.1	7.9	7.1	8.2	41.0	90	89	87	90
Soya control	20	18	16	9.0	8.0	6.9	8.4	40.5	90	89	89	86
10% Spirulina	19	17	15	8.8	7.7	7.0	7.9	40.2	89	88	88	91
20% Spirulina	20	18	16	8.8	7.8	7.0	8.0	41.4	90	89	87	90
30% Spirulina	20	17	15	9.2	8.4	7.6	8.3	40.6	85	88	91	90

F.I. = Fertility index
G.I. = Gestation index
V.I. = Viability index
L.I. = Lactation index

In the table corresponding to the second mating (Table 6) none of the indices shows any adverse effects of Spirulina as compared with the soya controls. The weights of the litters, for their part, do not differ between the treated groups and the control groups.

Study of sub-acute toxicity in the F_{3b} generation

All the batches of animals showed a similar general condition and behaviour. The faeces in all cases were of a dark colour, without any change of form and consistency.

The increase in weight of the rats treated with Spirulina, as can be seen from Table 7, were slightly lower than the soya controls, a difference which can be observed from the second week onwards. However this difference is not significant, and was equally consistent between the males and the females.

Table 8 shows that the male and female rats which ingested Spirulina did so in smaller quantities than the soya control.

However the conversion efficiency was very similar, varying in the case of the males from 0.37 to 0.38 and in the females from 0.27 to 0.28.

The haematological data, as can be seen in Table 9, shows no changes as compared with the controls, either in the males or the females.

TABLE 7. INCREASE IN WEIGHT OF RATS OF THE F_{3b} GENERATION FED WITH SPIRULINA

Diet	Body weight (g) at week:							
	0	2	4	6	8	10	12	13
				MALES				
Ordinary control	56	122	193	250	284	321	336	349
Soya control	57	121	190	248	282	315	332	344
10% Spirulina	56	119	186	245	279	311	336	338
20% Spirulina	57	118	185	243	276	308	333	335
30% Spirulina	57	119	185	244	275	308	332	336
				FEMALES				
Ordinary control	53	103	135	162	175	194	204	206
Soya control	54	102	133	159	171	189	196	202
10% Spirulina	53	102	131	157	169	186	194	197
20% Spirulina	53	101	129	154	167	182	193	195
30% Spirulina	53	102	130	153	166	181	191	193

TABLE 8. CONSUMPTION AND CONVERSION EFFICIENCY OF RATS OF THE F_{3b} GENERATION FED WITH SPIRULINA

Diet	Consumption of food (g/rat/day) during week						Efficiency*
	1	2	3	4	11	12	1st - 4th week
				MALES			
Ordinary control	8.7	11.7	14.6	15.9	16.8	17.3	0.38
Soya control	8.7	11.6	14.4	15.7	16.5	17.0	0.38
10% Spirulina	8.6	11.5	14.2	15.2	16.2	16.6	0.37
20% Spirulina	8.1	11.1	13.8	14.7	15.8	16.1	0.38
30% Spirulina	8.2	11.1	13.7	14.8	15.9	16.3	0.38
				FEMALES			
Ordinary control	7.7	10.6	11.1	11.7	12.3	12.6	0.28
Soya control	7.6	10.5	10.9	11.6	12.2	12.4	0.28
10% Spirulina	7.6	10.4	10.7	11.4	11.8	12.1	0.28
20% Spirulina	7.5	10.4	10.7	11.2	11.6	12.0	0.27
30% Spirulina	7.5	10.5	10.9	11.1	11.5	11.8	0.27

* gain in weight/food consumed

TABLE 9. HAEMATOLOGICAL PARAMETERS OF RATS OF THE F_{3b} GENERATION FED WITH SPIRULINA OVER A PERIOD OF 13 WEEKS

Treatment	Haemoglobin (g/100 ml)	Haematocrit (%)	Erythrocytes ($10^6/mm^3$)	Leucocytes				
				Total ($10^3/mm^2$)	Differential lymph.	neut.	eos.	mono.
MALES								
Ordinary control	14.5	48.4	7.3	13.7	82.8	14.3	2.7	0.2
Soya control	14.3	48.9	7.0	13.9	82.0	14.7	3.1	0.2
10% Spirulina	14.4	48.6	7.2	14.3	81.3	15.6	2.8	0.3
20% Spirulina	14.7	49.2	7.4	14.0	82.5	14.5	2.8	0.2
30% Spirulina	14.6	49.4	7.1	14.3	82.9	14.2	2.6	0.3
FEMALES								
Ordinary control	14.2	48.2	7.0	13.6	84.3	12.5	2.9	0.3
Soya control	14.2	48.5	6.8	13.2	84.0	12.9	2.8	0.3
10% Spirulina	14.1	48.1	6.9	13.8	84.2	13.1	2.4	0.3
20% Spirulina	14.3	48.3	7.1	12.7	83.7	13.7	2.4	0.2
30% Spirulina	14.3	48.8	6.7	13.6	84.7	12.4	2.6	0.3

In the same way the biochemical analyses on the serum do not show any changes due to Spirulina, as can be seen from an examination of Table 10.

Table 11 sets out the results of the analysis of the urine, which again shows no differences between the various groups.

Table 12 gives the data for the functional tests on the kidneys, determined by the excretion of phenol red, specific gravity and the glutamic-oxalacetic transaminase activity. A significant difference will be noted in the specific gravity of the male rats ingesting 20% of Spirulina and in the females of the ordinary control. There is a similarity in the glutamic-oxalacetic transaminase in the ordinary control males.

Furthermore the relative weight of the organs, the results of which are given in Table 13, show significant differences in the weight of the heart, kidney and seminal vesicles in the case of the males. In the case of the females some differences can be noted in the weight of the lungs and the spleen. In both cases the differences do not show any relationship with the dosage concerned, nor is it accompanied by any pathological differences which would indicate any possible toxicity of the alga.

The macroscopic study of the organs reveals cases of hydronephrosis in all batches, but the histopathological study did not indicate any special lesions which could be attributed to Spirulina and which would be qualitatively and quantitatively encountered in the control and treated animals (Table 14). Many of these lesions were the same as those encountered in the non-generation study of sub-acute toxicity.

TABLE 10. ANALYSIS OF THE SERUM OF RATS OF THE F_{3b} GENERATION FED WITH SPIRULINA FOR A PERIOD OF 13 WEEKS

Treatment	MALES				FEMALES			
	GOT (RFU)	GPT (RFU)	AP (BLU)	TSP g/100 ml	GOT (RFU)	GPT (RFU)	AP (BLU)	TSP g/100 ml
Ordinary control	161	31.6	5.6	6.2	152	23.2	4.3	6.8
Soya control	157	34.7	6.5	6.2	145	23.7	4.8	6.6
10% Spirulina	143	33.2	5.9	6.4	138	25.3	5.1	7.0
20% Spirulina	149	32.8	6.1	6.6	141	24.8	4.4	6.2
30% Spirulina	154	34.5	5.7	6.3	147	22.1	4.5	6.9

GOT = Glutamic-oxalacetic transaminase
GPT = Glutamic-pyruvic transaminase
AP = Alkaline phosphase
TSP = Total serum protein
RFU = Reitman-Frankel units
BLU = Bessey-Lowry units

TABLE 11. ANALYSIS OF THE URINE OF RATS OF THE F_{3b} GENERATION FED WITH SPIRULINA FOR A PERIOD OF 13 WEEKS

Treatment	Appearance	pH	glucose	protein	occult blood	ketones	Analysis of sediment					
							eryth.	leuc.	epith.	amorph.	cryst.	bact.
					MALES							
Ordinary control	yellow	6	–	+	–	–	–	–	+	+	–	+
Soya control	yellow	6	–	+	–	–	–	–	+	++	–	+
10% Spirulina	yellow	5	–	+	–	–	–	–	+	+	+	+
20% Spirulina	yellow	5	–	+	–	–	–	–	+	++	+	+
30% Spirulina	yellow	6	–	+	–	–	–	–	+	+	–	+
					FEMALES							
Ordinary control	yellow	5	–	+	–	–	–	–	+	++	–	+
Soya control	yellow	5	–	+	–	–	–	–	+	+	+	+
10% Spirulina	yellow	5	–	+	–	–	–	–	+	++	+	+
20% Spirulina	yellow	5	–	+	–	–	–	–	+	+	–	+
30% Spirulina	yellow	5	–	+	–	–	–	–	+	++	–	+

TABLE 12. RESULTS OF FUNCTIONAL TESTS ON THE KIDNEYS OF RATS OF THE F_{3b} GENERATION FED WITH SPIRULINA FOR A PERIOD OF 13 WEEKS

Diet	Excretion of phenol red (% in 1 hour)	Specific gravity	GOT (RFU)
		MALES	
Ordinary control	54.2	1.0552	15.6*
Soya control	56.3	1.0648	11.4
10% Spirulina	52.7	1.0656	10.9
20% Spirulina	55.5	1.0839**	11.2
30% Spirulina	57.4	1.0627	10.8
		FEMALES	
Ordinary control	62.8	1.0749*	12.6
Soya control	62.1	1.0537	9.2
10% Spirulina	60.9	1.0561	9.8
20% Spirulina	62.2	1.0653	11.4
30% Spirulina	64.0	1.0644	10.7

* $P < 0.05$; ** $P < 0.01$: by Student's test

TABLE 13. RELATIVE WEIGHTS OF THE ORGANS OF RATS OF THE F_{3b} GENERATION FED WITH SPIRULINA FOR A PERIOD OF 13 WEEKS

Treatment	Body weight	Heart	Brain	Lung	Kidney	Liver	Spleen	Testicles	Ovary	Seminal vesicle	Thymus	Thyroid	Adrenal	Pituitary
						MALES								
Ordinary control	349	0.388	0.47	0.38	0.80	3.47	0.174	0.93	-	0.318	0.096	0.0067	0.0192	0.0034
Soya control	344	0.402	0.52	0.35	0.76	3.42	0.170	0.84	-	0.325	0.098	0.0070	0.0195	0.0031
10% Spirulina	338	0.385	0.51	0.40	0.77	3.44	0.168	0.90	-	0.334	0.105	0.0069	0.0188	0.0036
20% Spirulina	335	0.431**	0.55	0.37	0.82	3.37	0.176	0.88	-	0.372**	0.087	0.0074	0.0207	0.0029
30% Spirulina	329	0.407	0.49	0.42	0.93*	3.44	0.173	0.86	-	0.386**	0.093	0.0072	0.0197	0.0035
						FEMALES								
Ordinary control	206	0,414	0,76	0.41	0.75	3.28	0.190	-	0.027	-	0.122	0.0071	0.0238	0.0040
Soya control	202	0.422	0.74	0.43	0.72	3.33	0.202	-	0.033	-	0.111	0.0074	0.0236	0.0038
10% Spirulina	197	0.408	0.76	0.47	0.68	3.15	0.213	-	0.030	-	0.118	0.0070	0.0255	0.0036
20% Spirulina	195	0.429	0.82	0.40	0.69	3.27	0.237**	-	0.032	-	0.124	0.0078	0.0242	0.0041
30% Spirulina	191	0.436	0.80	0.65*	0.74	3.12	0.199	-	0.029	-	0.128	0.0080	0.0263	0.0035

* $P < 0.05$; ** $P < 0.01$: by Student's test

TABLE 14. INCIDENCE OF HISTOPATHOLOGICAL OBSERVATIONS ON RATS OF THE F_{3b} GENERATION FED WITH SPIRULINA

Lesion	Diet*	Males					Females				
		A	B	C	D	E	A	B	C	D	E
	No. of rats examined	10	10	10	10	10	10	10	10	10	10
Kidneys											
Lymphoplasmocytaric infiltration with perivascular focus		1	1	1	0	0	0	0	1	1	0
Hyalinization of the arterial walls		0	0	0	1	0	1	0	0	0	0
Bilateral hydronephrosis		0	1	0	0	0	0	0	1	0	0
Intratubular hyaline cylinders		1	0	1	1	0	1	1	1	0	0
Liver											
Periportal lymphoplasmocytaric infiltration		0	0	1	1	0	0	0	0	0	1
Hyalinization of the arteriolar walls		1	1	0	0	1	1	0	0	1	0
Lungs											
Focal lymphoplasmocytaric infiltration		2	4	3	3	5	4	5	3	3	2
Vascular congestion		0	1	1	1	0	0	1	0	1	1
Calcareous deposits		0	0	1	0	0	0	1	0	0	0
Pituitary gland											
Cysts		0	0	0	1	0	0	0	1	0	1
Bladder											
Lymphoplasmocytaric infiltration		0	0	0	0	1	0	1	0	0	0
Testicles											
Periarteritis		0	1	1	0	0	0	0	0	0	0
Suprarenal glands											
Cortical hyperplasia		1	0	0	1	0	0	0	1	0	0
Heart											
Ventricular dilation		1	0	1	0	0	0	0	0	0	0

* Diet A - ordinary control: B - soya control: C - 10% Spirulina:
D - 20% Spirulina: E - 30% Spirulina

DISCUSSION

Analysis of the results of the reproduction and lactation study show that no adverse effect is produced on the fertility or gestation of rats which are fed with Spirulina at 10, 20 and 30%, as compared with the soya control.

However the corresponding indices reflect slight reductions as from the first matings of the F_{1b} generation; this is the same as with the soya and ordinary control groups, and is the reason why no particular action can be attributed to the product.

This reduction is also found between the first and second matings of the F_{2b} generation.

The values of the viability and lactation indices are reduced from the second mating of the F_o generation, and in the case of the first mating of the F_{1b} generation the lactation index falls to a value of 77. The two indices do, however, recover in the last two generations. In each of the two cases it is not possible to judge the effect of the alga, since this reduction is not found to show any relationship to the level of Spirulina; this is the case with the value of these indices throughout all the generations which show a slight increase in relation to the soya control.

The weight of the litters recorded at 4 and 21 days after birth shows no reduction, indicating that Spirulina does not cause any undesirable effects on this parameter.

As far as the study of sub-acute toxicity is concerned some changes are found in the F_{3b} generation in regard to behaviour, weight increase, consumption and conversion efficiency, haematological parameters and serum and urine analysis. Some differences which are statistically significant are found in the specific gravity of the urine and the glutamic-oxalacetic transaminase. However some of the weights of the organs differ significantly from the soya control. In neither case is there any relationship with the level of the alga, so that this can not be attributed to it. The absence of undesirable effects in the reproduction and lactation of rats fed with Spirulina at various levels is similar to the other results of the toxicity studies carried out with the alga.

STUDIES OF THE MUTAGENICITY OF SPIRULINA, CARRIED OUT ON RATS AND MICE

SUMMARY

Sprague Dawley rats and CD-1 mice were fed with 30% Spirulina on five days of the week for a period of three months. After this time the males were mated during one week with two females, which were then replaced by another two in the following week. This scheme was continued for a period of four weeks. At 15 or 16 days after the males had been introduced into the cage of the females the latter were sacrificed. The uterus was examined to count the live implantations, the dead implantations and the corpus luteus. The latter were not counted in the case of mice.

EXPERIMENTAL PART

Materials

Spirulina processed by the Sosa Texcoco Company was used, taken from a bulked production batch.

Animals

Sprague Dawley Rats from the Breeding Section of the Centro Médico Nacional and CD-1 mice from the Instituto Miles de Terapéutica were used.

Diets

They were fed with a diet containing 30% Spirulina and, as control, a diet based on soya, similar in both cases to those used in the other experiments.

Procedure

Male rats and mice of approximately 10 weeks of age and of proven fertility, were divided into two batches of ten animals, one of which received the diet for five days a week for 12 weeks.

During this time each male was mated with two females for a period of one week. At the end of this time the females were replaced by another two, and in this way four weeks of mating

were completed. The females were sacrificed by cervical dislocation 15 or 16 days after the introduction of the males and the uterus was examined, counting the number of live implantations, dead implantations and corpus luteus. The latter was not counted in the case of the mice.

RESULTS AND DISCUSSION

Tables 1 and 2 show the results for the rats and mice, from which it can be observed that Spirulina does not cause any changes in the parameters being considered.

These results agree with those of the multigeneration studies on reproduction and lactation, from which valid information in this respect can therefore be obtained.

The negative data of the present study are regarded as evidence that Spirulina does not have any mutagenic effects.

TABLE 1. RESULTS OF THE TEST OF LETHAL DOMINANTS IN RATS FED WITH SPIRULINA

Parameter	Soya control				30% Spirulina			
	Weeks							
	1	2	3	4	1	2	3	4
Number of:								
Females mated	18	18	19	18	20	20	20	18
Pregnant females	16	15	17	16	18	17	19	19
Implantations/pregnancies	11.7	10.6	11.5	10.7	10.4	11.2	10.6	11.1
Live implantations/pregnancies	10.8	9.6	10.7	9.8	9.6	10.5	10.0	10.2
Dead implantations/pregnancies	0.9	1.0	0.8	0.9	0.8	0.7	0.6	0.9

TABLE 2. RESULTS OF THE TEST OF LETHAL DOMINANTS IN MICE FED WITH SPIRULINA

Parameter	Soya control				30% Spirulina			
	Weeks							
	1	2	3	4	1	2	3	4
Number of:								
Females mated	20	20	19	20	20	20	19	19
Pregnant females	17	18	17	19	18	18	19	18
Implantations/pregnancies	11.1	10.4	10.9	10.3	10.9	10.2	10.8	10.4
Live implantations/pregnancies	10.3	9.7	9.9	9.5	10.5	9.6	9.9	9.3
Dead implantations/pregnancies	0.8	0.7	1.0	0.8	0.4	0.6	0.9	1.1

STUDIES ON THE TERATOGENICITY OF SPIRULINA, CARRIED OUT IN RATS, MICE AND HAMSTERS.

SUMMARY

Wistar rats, CD-1 mice and Dorado hamsters born to mothers fed Spirulina at levels of 10, 20 and 30% were used. Two batches were used as controls, one of these being fed with a diet normally used in the laboratory, the other with a diet based on soya. The rats were fed from the 7th to the 9th, from the 7th to the 14th, from the 1st to the 14th and from the 1st to the 21st day of gestation. The mice received the diets from the 7th to the 9th, from the 7th to the 13th, from the 1st to the 13th and from the 1st to the 19th days. Finally the hamsters were treated from the 7th to the 9th, the 7th to the 11th, the 1st to the 11th and the 1st to the 14th days. The females were checked for weight increase and notes were made of embryonic reabsorption on the day of sacrifice. The foetuses were examined to detect any internal and external malformations. The level of teratogenicity is expressed by means of a mean "teratogenic index". Spirulina does not cause malformations or embryonic reabsorption in any of the species at the levels and gestation periods used.

EXPERIMENTAL PART

Materials

The Spirulina used in this study was obtained from the pilot plant of Soso Texcoco S.A., using the process previously described.

Animals

Adult female and male Wistar rats donated by the Instituto Miles de Terapéutica, CD-1 mice provided by the Instituto de Higiene de la Secretaría de Salubridad y Asistencia, and hamsters of the Dorada race obtained from the Centro Médico Nacional del Instituto Mexicano del Seguro Social, were used.

Diets

For the study of teratogenesis five types of diet were used. Two served as controls, and consisted of one based on soya, the other being a commercial diet normally used in the laboratory. The experimental diets contained 10, 20 and 30% Spirulina which was gradually substituted for the soya. The diets were prepared approximately every three or four weeks and were stored at the temperature of the room.

Procedure

Before the commencement of the experiment and during it the animals were kept in rooms at temperatures of 22 $\pm$ 1°C with controlled cycles of light and humidity.

Food and water were provided "ad libitum" The rats were divided up by lot into groups of five females with which a male was left during the night. Mating was verified on the following day by the presence of spermatazoa in a vaginal smear. If spermatazoa were detected this was considered to be the first day of gestation.

In the case of the mice, which were also formed into groups of five females, mating was verified by the presence of the sperm plug in the vagina of the females, observed however on the day following the male and female contacts, which was regarded as being the first day.

For the mating of the hamsters the females were placed in a cage in which a male lived at 20.00 hours. If the female showed lordosis it was covered by the male and was left for the night. The following day was considered as being the first day of gestation.

The mated rats, mice and hamsters were divided up respectively by random into five batches each of which received one of the indicated diets.

In the case of the rats the diet was given to them from the 7th to the 9th, from the 7th to the 14th, from the 1st to the 14th and from the 1st to the 21st day of gestation. For their part the mice received the diets from the 7th to the 9th, from the 7th to the 13th, from the 1st to the 13th and from the 1st to the 19th day; finally the hamsters received the diet from the 7th to the 9th, from the 7th to the 11th, from the 1st to the 11th and from the 1st to the 14th day of gestation.

Two types of control were made on the females, relating to the development of weight during gestation and the identification of the points of implantation in the uterine walls on the day of sacrifice which, in the case of the rats, was the 21st day of gestation, in the mice the 19th day, and in the hamsters the 14th day: in each case this was done by inhalation of chloroform.

The females were weighed on the first day of gestation and on the day of being sacrificed. In addition intermediate weighings were carried out in the case of the rats on the 7th and 14th day, in the mice on the 6th and 13th day and in the hamster on the 6th and 10th day of gestation.

Immediately after the animals had been sacrificed a caesarian was carried out to extract the foetuses which were counted and noted as living or dead. Subsequently they were subjected to minute examination with the purpose of detecting any malformations. For the study of internal malformations the Wilson technique of a series of selections (1964) was used, including the head, thorax and abdomen. This procedure was used on approximately one-third of the foetuses. For the study of the skeleton recourse was had to the alizarine dyeing method, after Dawson (1926) and as modified by Staples (1964), and this was applied to two-thirds of the total number of foetuses.

The count of the implantations in the uterine walls, to determine embryonic reabsorption, was carried out using the Salewski (1964) technique.

When processing and interpreting the results account was taken firstly of the number of mothers presenting abnormal foetuses or embryonic reabsorptions and secondly the number of abnormal foetuses and reabsorptions in relation to the number of implantations.

Using this control criterion a series of tables has been produced for each species, corresponding respectively to: increase in weight, absolute values for the mothers, absolute values for the foetuses, percentages for the mothers, percentages for the foetuses, teratogenic index and description of the abnormalities.

In the tables of percentages for the foetuses there are also included, independently, the mean foetal weight, the number of implantations for each fertile female and the number of foetuses for each pregnant mother. These three parameters were treated statistically for significant differences using the "t" test.

The teratogenic index for the mothers and the foetuses was calculated, on the basis of the average percentages, using the following formula; (Chamorro, 1972):

$$\frac{\text{Controls affected - experimental animals affected}}{\text{100 - normal controls}} \times 100$$

RESULTS

Rats

Weight increase

The results for the consumption of Spirulina against the increase in weight of pregnant rats, ingested over various periods, are shown in figures 1 - 4.

Figure 1, covering the treatment of animals over the 7th and 9th days of gestation, shows that Spirulina does not cause any variation in body weight in relation to control animals, either during treatment or once this had been terminated. The curve corresponding to the 30% level shows an increase which is slightly less rapid than in the other groups, but this can not be held to be significant, however.

The weight increase in rats treated during organogenesis, the results of which are shown in Figure 2, do not show an effect from the Spirulina. After commencement of treatment on the 7th day the growth follows a trajectory which is similar in all the groups.

Those animals which received the alga from the 1st to 14th day of gestation (Fig. 3), do not show any difference as compared with the soya control. The group which received 20% increased slightly less rapidly than the remaining ones from the beginning of treatment but, once this was terminated, they tended to be similar to the other groups.

Rats which were fed during the whole of the gestation period (Fig. 4) showed no changes in growth. It was, however, observed that the group at 10% Spirulina grew, from the 7th day onwards, at a rate which was slightly lower than the soya control but, as in the previous case, they had recovered at the end of the treatment.

The 30% level however produced an increase which was slightly more rapid. This increase was shown from the first third of the gestation up to the end of it. The difference against the soya control was not, however, greater than 10%.

Fig.1 WEIGHT INCREASE IN RATS TREATED WITH SPIRULINA FROM THE 7th TO THE 9th DAY OF GESTATION

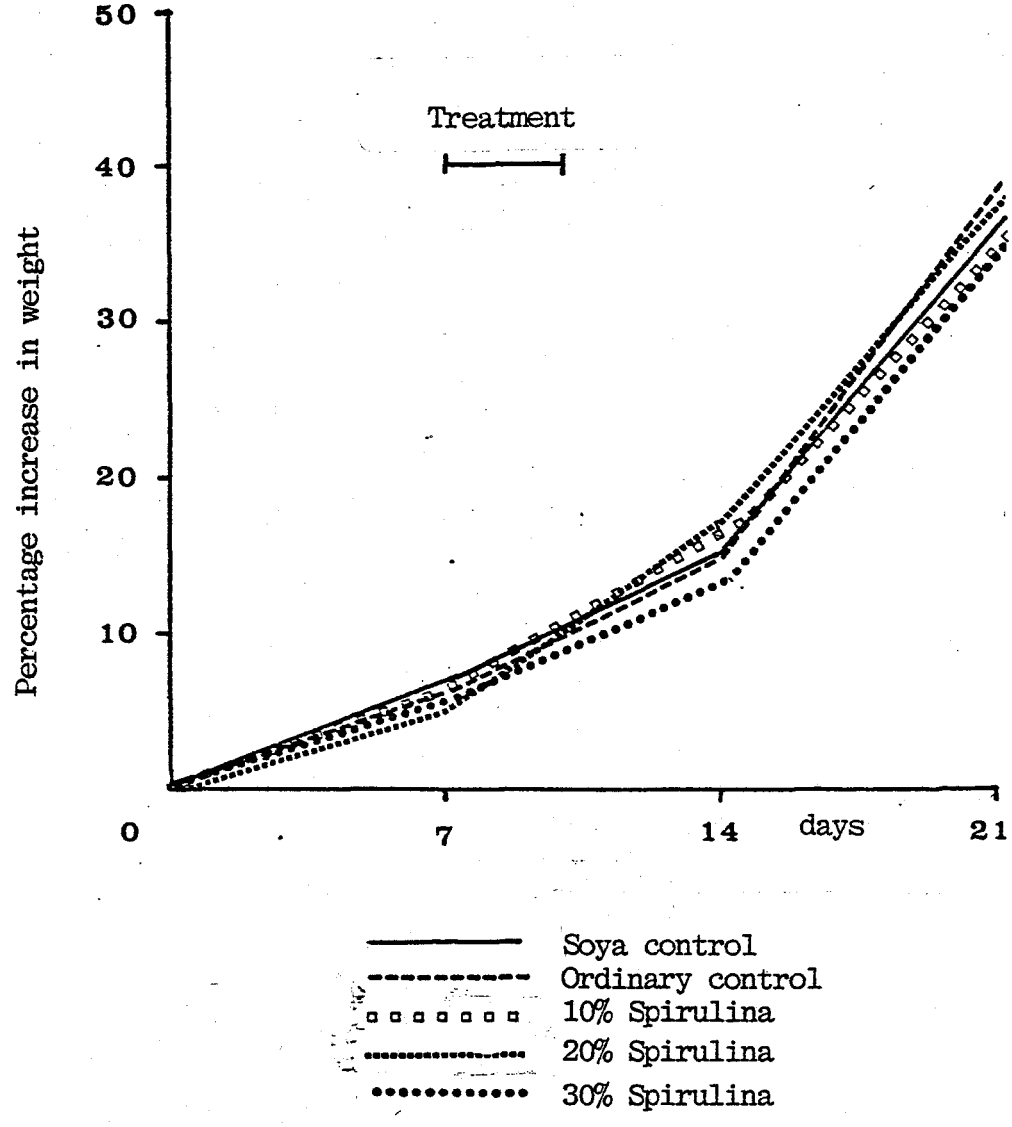


Fig.2 WEIGHT INCREASE IN RATS TREATED WITH SPIRULINA FROM THE 7th TO THE 14th DAY OF GESTATION

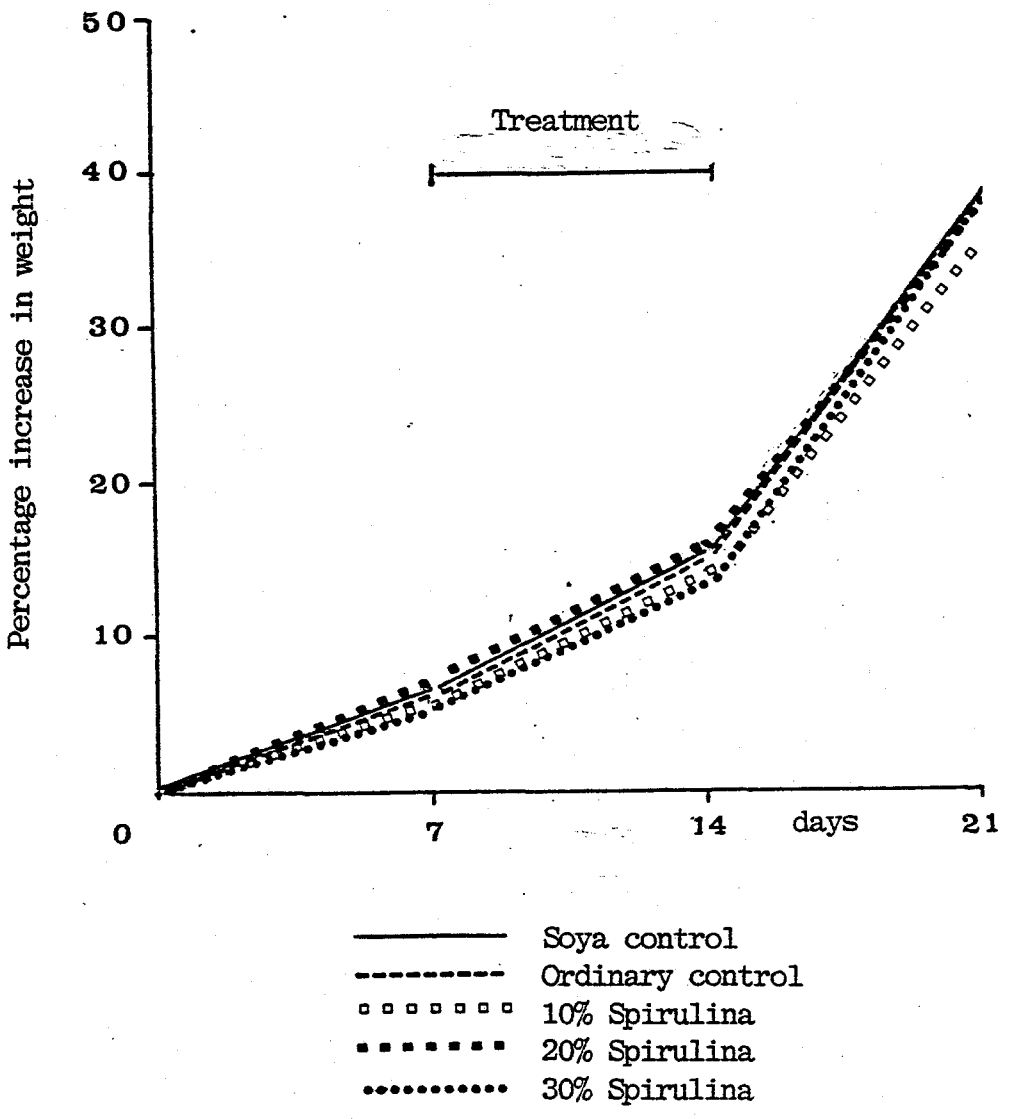


Fig.3 WEIGHT INCREASE IN RATS TREATED WITH SPIRULINA FROM THE 1st TO THE 14th DAY OF GESTATION

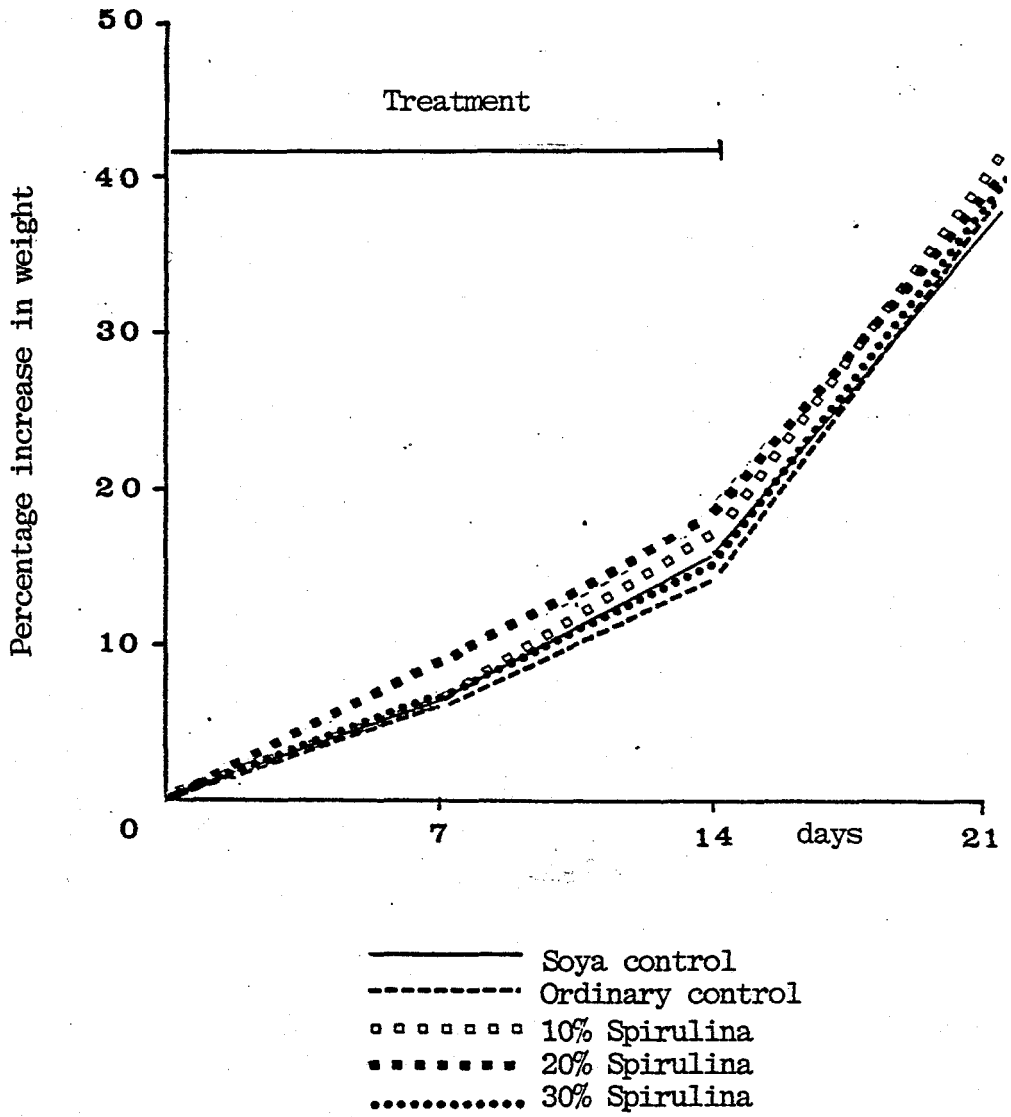
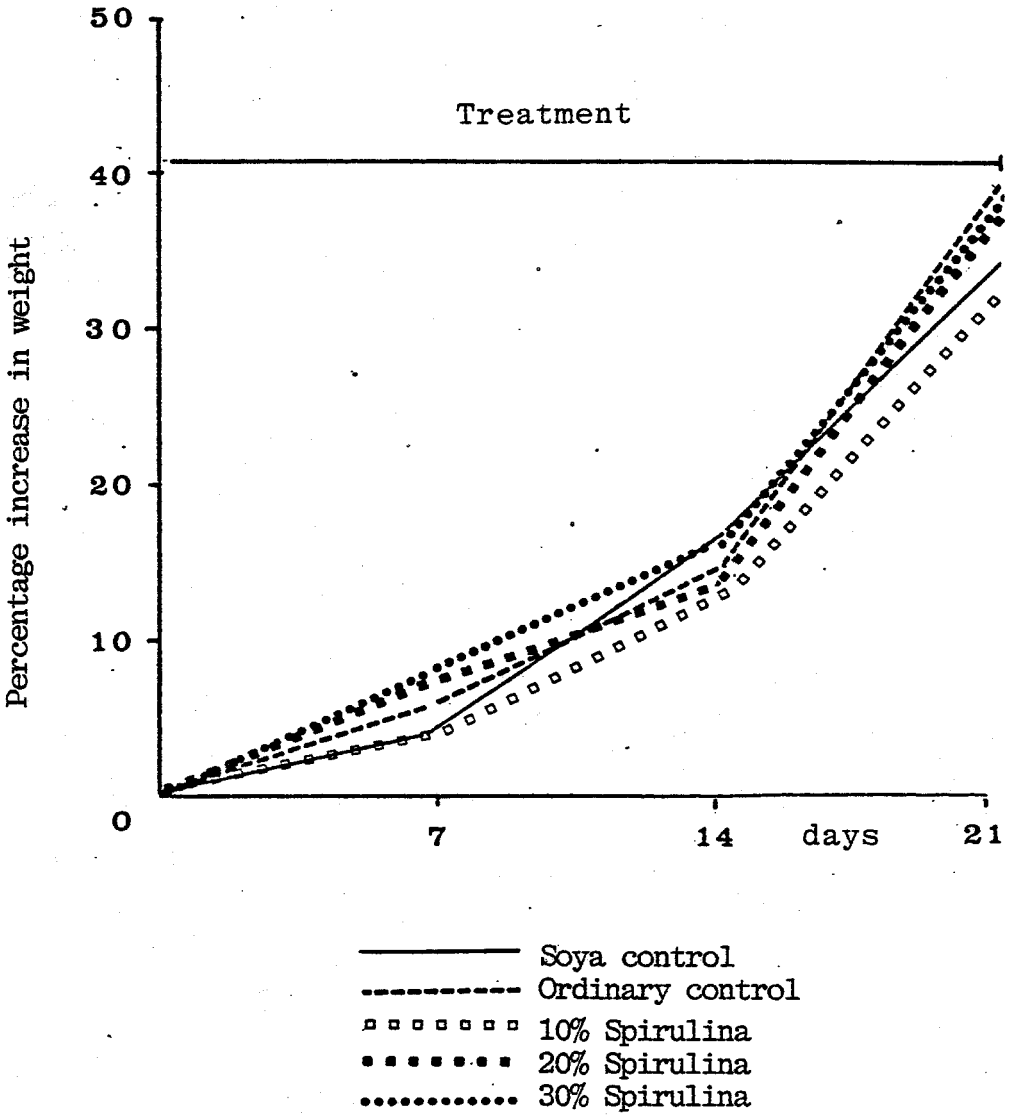


Fig.4 WEIGHT INCREASE IN RATS TREATED WITH SPIRULINA FROM THE 1st TO THE 14th DAY OF GESTATION



Teratogenic effect

Tables 1 to 4 give the absolute figures for the mothers; these are termed the "calculation bases". These show firstly the number of mated females (1) that is to say those females in which mating was proved by the presence of spermatazoa in the vaginal smear. (In the case of mice by the spermatic plug, in the hamsters by visual evaluation). Then the fertile females are considered (2), that is to say those in which nidation had been produced, indicated by the presence of foetuses or by the existence of implantations in the uterine walls, revealed by dyeing using the Salewski method.

Within the fertile females are then differentiated those females with normal litters (2.1) and those with affected litters (2.2). The mothers with normal litters are those which show no malformations and evidence of a maximum of one embryonic reabsorption. Females with affected litters (2.2) are then subdivided into females showing more than one reabsorbed foetus or dead foetuses (2.2.1); females showing only abnormal foetuses (2.2.2) and females showing simultaneously reabsorbed, dead and abnormal foetuses (2.2.3). Under 3 are included those which, at the time of sacrifice, bore at least one foetus.

Tables 5 to 8 show the "calculation bases" for the foetuses. These show the number of implantations (1) and the number of foetuses (2), which are then divided into normal (2.1) and affected (2.2), the latter being subdivided into those which showed abnormalities (2.2.1) or which were reabsorbed or dead (2.2.2).

TABLE 1. THE EFFECT OF SPIRULINA ON RATS, INGESTED FROM THE 7th TO THE 9th DAY OF GESTATION. CALCULATION BASES

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of females:					
1 - Mated	27	27	22	29	26
2 - Fertile, with:	24	26	19	27	23
2.1 Normal litters	17	20	13	21	19
2.2 Litters affected by:	7	6	6	6	4
2.2.1 Reabsorbed or dead foetuses	4	0	1	1	0
2.2.2 Abnormal foetuses	1	3	1	1	1
2.2.3 Reabsorbed or dead and abnormal foetuses	2	3	4	4	3
3 - Gestating at time of sacrifice	23	26	18	26	23

TABLE 2. EFFECT OF SPIRULINA ON RATS, INGESTED FROM THE 7th TO THE 14th DAY OF GESTATION. CALCULATION BASES

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of females:					
1 - Mated	27	22	21	24	22
2 - Fertile, with:	24	19	20	22	21
2.1 Normal litters	17	15	15	16	16
2.2 Litters affected by:	7	4	5	6	5
2.2.1 Reabsorbed or dead foetuses	4	1	2	1	2
2.2.2 Abnormal foetuses	1	2	2	2	1
2.2.3 Reabsorbed or dead and abnormal foetuses	2	1	1	3	2
3 - Gestating at time of sacrifice	23	19	19	20	20

TABLE 3. EFFECT OF SPIRULINA ON RATS, INGESTED FROM THE 1st TO THE 14th DAY OF GESTATION. CALCULATION BASES

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of females:					
1 - Mated	27	26	25	25	25
2 - Fertile, with:	24	25	20	24	22
2.1 Normal litters	17	16	15	17	16
2.2 Litters affected by:	7	9	5	7	6
2.2.1 Reabsorbed or dead foetuses	4	8	3	3	1
2.2.2 Abnormal foetuses	1	1	1	4	5
2.2.3 Reabsorbed or dead and abnormal foetuses	2	0	1	0	0
3 - Gestating at time of sacrifice	23	25	20	24	22

TABLE 4. EFFECT OF SPIRULINA ON RATS, INGESTED FROM THE 1st TO THE 21st DAY OF GESTATION. CALCULATION BASES

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of females:					
1 - Mated	27	25	26	28	23
2 - Fertile, with:	24	22	23	25	23
2.1 Normal litters	17	15	16	16	15
2.2 Litters affected by:	7	7	7	9	8
2.2.1 Reabsorbed or dead foetuses	4	3	3	6	5
2.2.2 Abnormal foetuses	1	3	3	2	2
2.2.3 Reabsorbed or dead and abnormal foetuses	2	1	1	1	1
3 - Gestating at time of sacrifice	23	22	21	23	21

TABLE 5. EFFECT OF SPIRULINA ON RATS, INGESTED FROM THE 7th TO THE 9th DAY OF GESTATION. CALCULATION BASES

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of:					
1. Implantations	244	270	202	267	219
2. Foetuses	212	243	178	235	198
2.1. Normal	209	235	172	230	194
2.2. Affected by:	35	35	30	37	25
2.1.1. Abnormalities	3	8	6	5	4
2.2.2. Reabsorption or death	32	27	24	32	21

TABLE 6. EFFECT OF SPIRULINA ON RATS, INGESTED FROM THE 7th TO THE 14th DAY OF GESTATION. CALCULATION BASES

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of:					
1. Implantations	244	195	210	218	223
2. Foetuses	212	175	187	195	195
2.1. Normal	209	169	180	190	190
2.2. Affected by:	35	26	30	28	33
2.2.1. Abnormalities	3	6	7	5	5
2.2.2. Reabsorption or death	32	20	23	23	28

TABLE 7. EFFECT OF SPIRULINA ON RATS, INGESTED FROM THE 1st TO THE 14th DAY OF GESTATION. CALCULATION BASES

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of:					
1. Implantations	224	284	201	241	219
2. Foetuses	212	240	178	224	209
2.1. Normal	209	239	176	219	203
2.2. Affected by:	35	45	25	22	16
2.2.1. Abnormalities	3	1	2	5	6
2.2.2. Reabsorption or death	32	44	23	17	10

TABLE 8. EFFECT OF SPIRULINA ON RATS, INGESTED FROM THE 1st TO THE 21st DAY OF GESTATION. CALCULATION BASES

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of:					
1. Implantations	244	230	211	244	217
2. Foetuses	212	193	180	182	177
2.1. Normal	209	189	176	179	175
2.2. Affected by:	35	41	35	65	42
2.2.1. Abnormalities	3	4	4	3	2
2.2.2. Reabsorption or death	32	37	31	62	40

Tables 9 to 12 give the previous data converted into percentages and in regard to the mothers.

Table 9 shows that the percentages of the mothers which are affected are similar in the groups treated with Spirulina and with the soya control. However there is a major difference between the two controls themselves. The mothers showing affected litters are distributed impartially amongst those which show only reabsorbed or dead foetuses, malformed or simultaneously reabsorbed or dead and malformed foetuses.

Table 10 indicates, however, that the percentage of litters which are affected are very similar to the soya control. As in the previous case the incidents are impartially distributed between the three classifications.

Table 11 shows that the percentages of those affected by Spirulina are, in all cases, smaller than the soya control group. In the case of the soya these are represented principally by females with reabsorbed foetuses. It is however also the case with the ordinary control and the group with 10% Spirulina. By contrast with levels of 20% and 30% the affected mothers are those which show principally abnormal foetuses, secondly those showing reabsorbed foetuses.

Table 12 again shows percentages of affected mothers amongst those treated with alga and those not treated which are very similar. In the ordinary control, and also at the 20% and 30% levels, the affected mothers are characterized principally by showing only reabsorbed or dead foetuses.

TABLE 9. SPIRULINA IN RATS, INGESTED FROM THE 7th TO THE 9th DAY OF GESTATION. PERCENTAGES

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
% of females:					
2. Fertile, with:	88.8	96.2	86.3	93.1	86.6
2.1 Normal litters	70.8	77.0	68.4	77.8	82.6
2.2 Litters affected by:	29.2	23.0	31.6	22.2	17.4
2.2.1 Reabsorbed or dead foetuses	16.7	0.0	5.3	3.7	0.0
2.2.2 Abnormal foetuses	4.2	11.5	5.3	3.7	4.3
2.2.3 Reabsorbed or dead and abnormal foetuses	8.3	11.5	21.0	14.8	13.0
3. Gestating at time of sacrifice	95.8	100.0	94.7	96.3	100.0

TABLE 10. SPIRULINA IN RATS, INGESTED FROM THE 7th TO THE 14th DAY OF GESTATION. PERCENTAGES.

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
% of females:					
2. Fertile, with:	88.9	86.3	95.2	91.7	95.4
2.1 Normal litters	70.8	78.9	75.0	72.7	76.2
2.2 Litters affected by:	29.2	21.0	25.0	27.3	23.8
2.2.1 Reabsorbed or dead foetuses	16.7	5.2	10.0	4.5	9.5
2.2.2 Abnormal foetuses	4.2	10.5	10.0	9.1	4.8
2.2.3 Reabsorbed or dead and abnormal foetuses	8.3	5.2	5.0	13.6	9.5
3. Gestating at time of sacrifice	95.8	100.0	95.0	90.9	95.2

TABLE 11. SPIRULINA IN RATS, INGESTED FROM THE 1st TO THE 14th DAY OF GESTATION. PERCENTAGES

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
% of females					
2. Fertile, with:	88.8	96.1	80.0	96.0	88.0
2.1. Normal litters	70.8	64.0	75.0	70.8	72.7
2.2. Litters affected by:	29.2	36.0	25.0	29.2	27.3
2.2.1. Reabsorbed or dead foetuses	16.7	32.0	15.0	12.5	4.5
2.2.2. Abnormal foetuses	4.2	4.0	5.0	16.7	22.7
2.2.3. Reabsorbed or dead and abnormal foetuses	8.3	0.0	5.0	0.0	0.0
3. Gestating at time of sacrifice	95.8	100.0	100.0	100.0	100.0

TABLE 12. SPIRULINA IN RATS, INGESTED FROM THE 1st TO THE 21st DAY OF GESTATION. PERCENTAGES

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
% of females:					
2. Fertile, with:	88.8	88.0	88.5	89.3	100.0
2.1. Normal litters	70.8	68.2	69.6	64.0	65.2
2.2. Litters affected by:	29.2	31.8	30.4	36.0	34.8
2.2.1. Reabsorbed or dead foetuses	16.7	13.6	13.0	24.0	21.7
2.2.2. Abnormal foetuses	4.2	13.6	13.0	8.0	8.7
2.2.3. Reabsorbed or dead and abnormal foetuses	8.3	4.5	4.3	4.0	4.3
3. Gestating at time of sacrifice	95.8	100.0	91.3	92.0	91.3

Tables 13 to 16 show the data relative to the foetuses. In the first of these it will be observed that there is no difference in affected foetuses between the soya control batch and those fed with Spirulina. Those affected were characterized primarily by reabsorption.

As far as the mean weight of the litters is concerned a significant difference is observed with 10 and 20% of Spirulina.

Table 14 however shows a major difference between the control and those treated; the affected foetuses, however, are all reabsorptions.

Table 15 shows that the incidence of foetuses affected by the ingestion of Spirulina are in all cases smaller than those encountered in the soya batch. There is a significant difference in the number of implantations per fertile female with the three levels of Spirulina, and there is also a difference in the mean weight of the foetuses corresponding to mothers treated with 30% Spirulina.

Table 16 indicates that the incidents are essentially embryonic reabsorptions which, in the case of the 20% Spirulina, account for 25.4%. However the affected foetuses, whether involving abnormalities or reabsorptions, do not differ radically. It will be noted however that there is a significant difference in the mean weight of those foetuses whose mothers received 10 and 30% of the alga, as is also the case between the controls.

TABLE 13. SPIRULINA IN RATS, INGESTED FROM THE 7th TO THE 9th DAY OF GESTATION. PERCENTAGES

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
% of foetuses					
2.1. Normal	85.6	87.0	85.1	86.1	88.6
2.2. Affected by:	14.3	13.0	14.8	13.8	11.4
2.2.1. Abnormalities	1.2	3.0	3.0	1.9	1.8
2.2.2. Reabsorptions or death	13.1	10.0	11.8	11.9	9.6
Mean of:					
- foetal weight (g)	3.2	3.4	3.0*	3.0*	3.5
- implantations per fertile female	10.1	10.3	10.6	9.9	9.5
- foetuses per pregnant female	9.2	9.3	9.8	9.0	8.6

TABLE 14. SPIRULINA IN RATS, INGESTED FROM THE 7th TO THE 14th DAY OF GESTATION. PERCENTAGES

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
% of foetuses :					
2.1. Normal	85.6	86.7	85.7	87.1	85.2
2.2. Affected by:	14.3	13.3	14.3	12.8	14.8
2.2.1. Abnormalities	1.2	3.1	3.3	2.3	2.2
2.2.2. Reabsorptions or death	13.1	10.2	10.9	10.5	12.5
Mean of:					
- foetal weight (g)	3.21	3.20	3.20	3.32	3.28
- implantations per fertile female	10.1	10.2	10.5	9.9	10.6
- foetuses per pregnant female	9.2	9.2	9.8	9.7	9.7

TABLE 15. SPIRULINA IN RATS, INGESTED FROM THE 1st TO THE 14th DAY OF GESTATION. PERCENTAGES

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
% of foetuses:					
2.1. Normal	85.6	84.1	87.6	90.9	92.7
2.2. Affected by:	14.3	15.8	12.4	9.1	7.3
2.2.1. Abnormalities	1.2	0.3	1.0	2.0	2.7
2.2.2. Reabsorptions or death	13.1	15.5	11.4	7.0	4.6
Mean of:					
- foetal weight (g)	3.21	3.14	3.18	3.12	3.28*
- implantations per fertile female	10.1	11.3	10.0*	10.0*	9.9*
- foetuses per pregnant female	9.2	9.6	8.9	9.3	9.2

TABLE 16. SPIRULINA IN RATS, INGESTED FROM THE 1st TO THE 21st DAY OF GESTATION. PERCENTAGES

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
% of foetuses:					
2.1. Normal	85.6	82.2	83.4	73.4	80.6
2.2. Affected by:	14.3	17.8	16.6	26.6	19.3
2.2.1. Abnormalities	1.2	1.7	1.9	1.2	0.9
2.2.2. Reabsorptions or death	13.1	16.1	14.7	25.4	18.4
Mean of:					
- foetal weight (g)	3.21*	3.04	3.20*	2.90	3.20*
- implantations per fertile female	10.1	9.5	9.1	9.7	9.4
- foetuses per pregnant female	9.2	8.7	8.5	7.9	8.4

Tables 17 to 20 refer to the values of the teratogenic index as obtained from the previous percentages.

As will be observed from table 17 this index is negative in the case of the mothers in relation to the soya control, which itself is negative in respect to the ordinary control which is not shown in this table.

The results for the foetuses in the same table also indicate that the alga has no teratogenic effect at any level.

Table 18, which shows the results of the treatment during organogenesis, also shows no teratogenic effect from Spirulina, either in the case of the mothers or in the case of the foetuses.

The same type of results are observed from table 19 in which they are all negative, with the exception of the data relating to the soya control.

Table 20 shows negative indices for the case of the 10% Spirulina and positive results for the other levels, whereas between the latter it scarcely amounts to 10.7 with 20% of alga.

TABLE 17. SPIRULINA IN RATS, INGESTED FROM THE 7th TO THE 9th DAY OF GESTATION. TERATOGENIC INDICES

% Spirulina	In the mothers	In the foetuses
10	- 11.2	2.0
20	- 1.3	0.9
30	- 7.3	- 1.8
Soya control	- 8.7	- 1.5

TABLE 18. SPIRULINA IN RATS, INGESTED FROM THE 7th TO THE 14th DAY OF GESTATION. TERATOGENIC INDICES

% Spirulina	In the mothers	In the foetuses
10	5.0	1.1
20	8.0	- 5.8
30	3.5	1.7
Soya control	-11.6	- 1.2

TABLE 19. SPIRULINA IN RATS, INGESTED FROM THE 1st TO THE 14th DAY OF GESTATION. TERATOGENIC INDICES

% Spirulina	In the mothers	In the foetuses
10	- 17.2	- 4.0
20	- 10.6	- 8.0
30	- 13.6	- 10.1
Soya control	9.6	1.7

TABLE 20. SPIRULINA IN RATS, INGESTED FROM THE 1st TO THE 21st DAY OF GESTATION. TERATOGENIC INDICES

% Spirulina	In the mothers	In the foetuses
10	- 2.0	- 1.4
20	6.1	10.7
30	4.4	1.8
Soya control	3.7	4.1

Tables 21 to 24 describe the malformed foetuses, resulting from the various treatments on the basis of external examination and analysis of the skeleton and viscera.

Table 21 shows cases of macerated foetuses in all the groups, foetuses which show haematomas and one foetus with generalized oedema fromthe 20% diet.

Examination of the skeleton, using colouration with alizarine, reveals a very limited number of foetuses with defects in the ribs in almost all the groups and with 30% of Spirulina, and one case of general retarded ossification.

The Wilson serial sections provide evidence of cases of hydronephrosis; this malformation is however frequent in the breed of animal used, and has been seen in previous experiments.

Table 22 shows that the external anomalies are cases of haematomas, whilst the levels of 10 and 20% each result in one case of generalized oedema.

Analysis of the skeleton shows various malformations, some of which are however encountered in the soya control; this is the case with those foetuses which show malformed ribs and poor cranial ossification; these are also repeated in the case of treatment with 10 and 20% of Spirulina.

The Wilson sections show cases of hydronephrosis and ectopia of the testicles.

Table 23 lists the lesions found in foetuses produced by mothers treated from the 1st to the 14th day of gestation. The abnormal foetuses encountered after the treatment with Spirulina refer to isolated cases of celesomia and oedema with micrognatia, not encountered in the soya control. On the other hand skeletal malformations, affecting the ribs in the controls and treated animals, and hydronephrosis amongst the visceral malformations, appear to be common in the experimental groups and in the soya control.

Table 24 lists the abnormalities found in foetuses whose mothers were treated during the whole of the gestation period. It will be seen that some malformations are similar to those encountered with the controls, and which are also shown at skeletal level by cases of hydrocephalus and hydronephrosis. Two cases of celosomia and a foetus with generalized oedema were found as distinct malformations in the controls. The number and frequency of the abnormal foetuses is, however, similar in the control and experimental groups.

TABLE 21. DESCRIPTION OF THE ABNORMAL FOETUSES PRODUCED BY RATS TREATED WITH SPIRULINA FROM THE 7th TO THE 9th DAY OF GESTATION

Type of examination	Ordinary control	Soya control	% Spirulina 10	20	30
EXTERNAL	1f. macerated	2f. macerated 1f. haematoma	2f. macerated	2f. macerated 1f. generalized oedema 1f. faulty development	1f. macerated 1f. haematoma
+	212	243	178	235	198
ALIZARINE	1f. malformed ribs	1f. malformed ribs 2f. reduction 13th ribs	3f. malformed ribs	1f. reduction 13th ribs	1f. general retarded ossification
+	141	164	118	152	127
WILSON	1f. unilateral hydronephrosis	2f. unilateral hydronephrosis	1f. unilateral hydronephrosis		1f. unilateral hydronephrosis
+	71	79	60	83	71

+ Number of foetuses examined.

TABLE 22. DESCRIPTION OF THE ABNORMAL FOETUSES PRODUCED BY RATS TREATED WITH SPIRULINA FROM THE 7th TO THE 14th DAY OF GESTATION

Type of examination	Ordinary control	Soya control	% Spirulina 10	20	30
EXTERNAL	1f. macerated	2f. haematoma	2f. haematoma 1f. generalized oedema	1f. generalized oedema	1f* haematoma
+	212	174	179	180	176
ALIZARINE	1f. malformed ribs	2f. malformed ribs 1f. poor craneal ossifn.	2f. general retarded ossification 1f* poor craneal ossifn.	1f. malformed ribs 2f. reduction of 13th ribs	2f. general retarded ossification 2f. extra ribs
+	141	113	121	125	114
WILSON	1f. unilateral hydronephrosis	1f. displacement of testicles	1f. bilateral hydronephrosis 1f* unilateral hydrocephalus	1f. bilateral hydronephrosis	1f* displacement of testicles
+	71	61	58	55	62

+ Number of foetuses examined.

* Same foetus.

TABLE 23. DESCRIPTION OF THE ABNORMAL FOETUSES PRODUCED BY RATS TREATED WITH SPIRULINA FROM THE 1st TO THE 14th DAY OF GESTATION

Type of examination	Ordinary control	Soya control	% Spirulina 10	20	30
EXTERNAL	1f. macerated		1f. celosomia 1f. haematoma	1f. haematoma	1f. oedema and micrognathia
+	212	240	178	224	200
ALIZARINE	1f. malformed ribs	1f. fused ribs		2f. extra ribs 1f. deformed ribs	2f. deformed ribs 1f. fused ribs
+	141	162	120	148	139
WILSON	1f. unilatera-hydronephrosis			1f. bilateral hydronephrosis	1f. bilateral hydronephrosis 1f. bilateral hydronephrosis and unilateral hydrocephalus
+	71	78	58	76	70

+ Number of foetuses examined.

TABLE 24. DESCRIPTION OF THE ABNORMAL FOETUSES PRODUCED BY RATS TREATED WITH SPIRULINA FROM THE 1st TO THE 21st DAY OF GESTATION

Type of examination	Ordinary control	Soya control	% Spirulina 10	20	30
EXTERNAL	1f. macerated		1f. celosomia 1f. deformed extremities	2f. celosomia	1f. generalized oedema
+	212	223	180	182	177
ALIZARINE	1f. malformed ribs	2f. reduction of 13th ribs 1f. malformed ribs	1f. malformed ribs	1f. extra ribs	
+	140	148	119	122	121
WILSON	1f. unilateral hydronephrosis	1f. bilateral hydrocephalus	1f. unilateral hydrocephalus		1f. unilateral hydronephrosis
+	72	75	61	60	56

+ Number of foetuses examined.

MICE

Weight increase

The figures and tables set out in the following pages for the results with mice show the same pattern as with the rats.

Figures 5 to 8 show the curves of increase in weight when Spirulina was fed over four different periods.

Figures 5 and 6 show that Spirulina does not modify the growth of mice either during the period of treatment itself or after this has been terminated.

In the case of ingestion from the 1st to the 13th day (Figure 7) it will be noted that the weight of all the groups of animals is slightly lower than the soya control during the period of treatment, but subsequently they show clear recovery.

Figure 8 shows, however, that during the first two-thirds of gestation there is a slight reduction in weight with respect to the soya control, but in the last third the groups with 10, 20 and 30% show a slight increase.

Fig.5 WEIGHT INCREASE IN MICE TREATED WITH SPIRULINA FROM THE 7th TO THE 9th DAY OF GESTATION

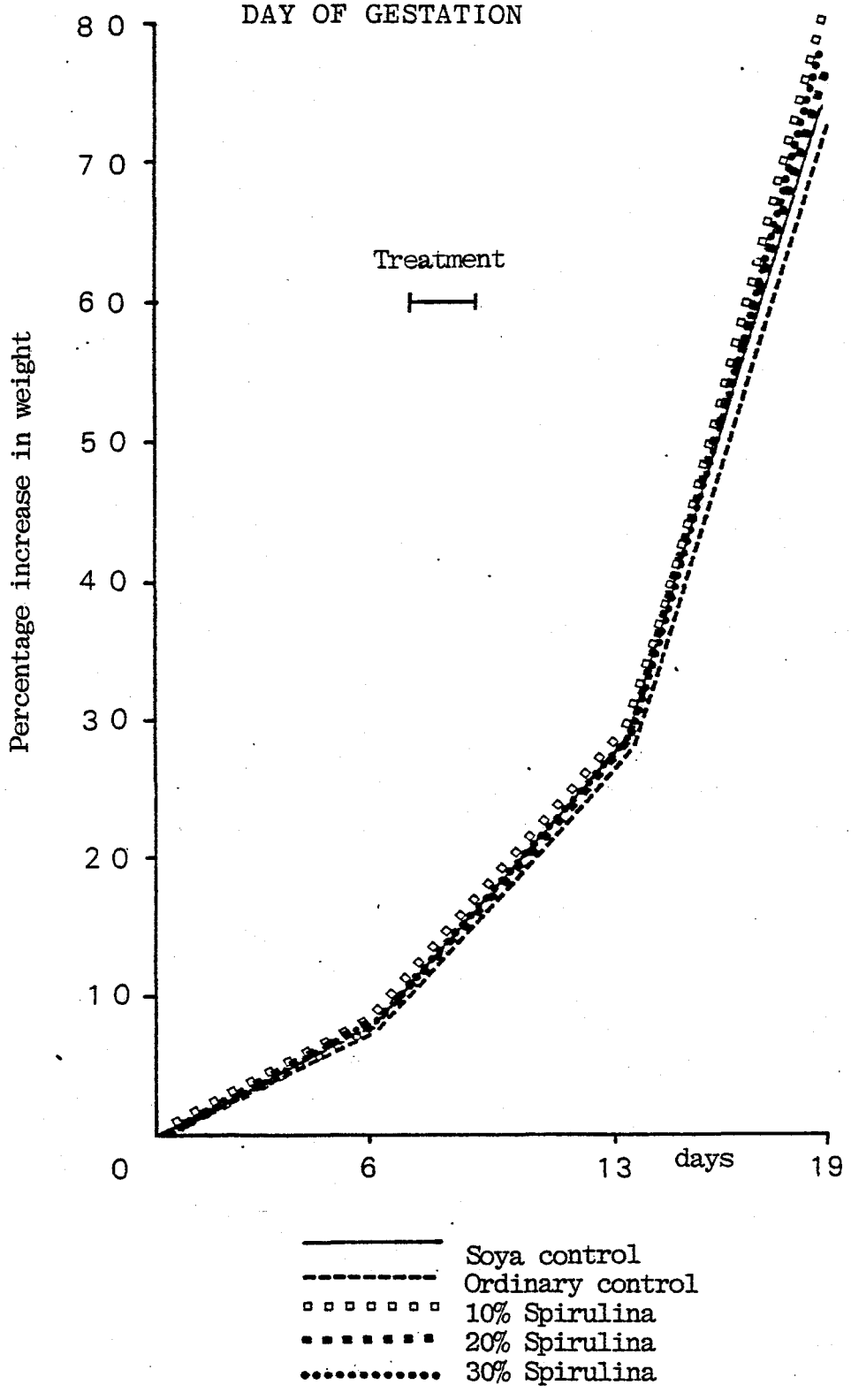


Fig. 6 WEIGHT INCREASE IN MICE TREATED WITH SPIRULINA FROM THE 7th TO THE 13th DAY OF GESTATION

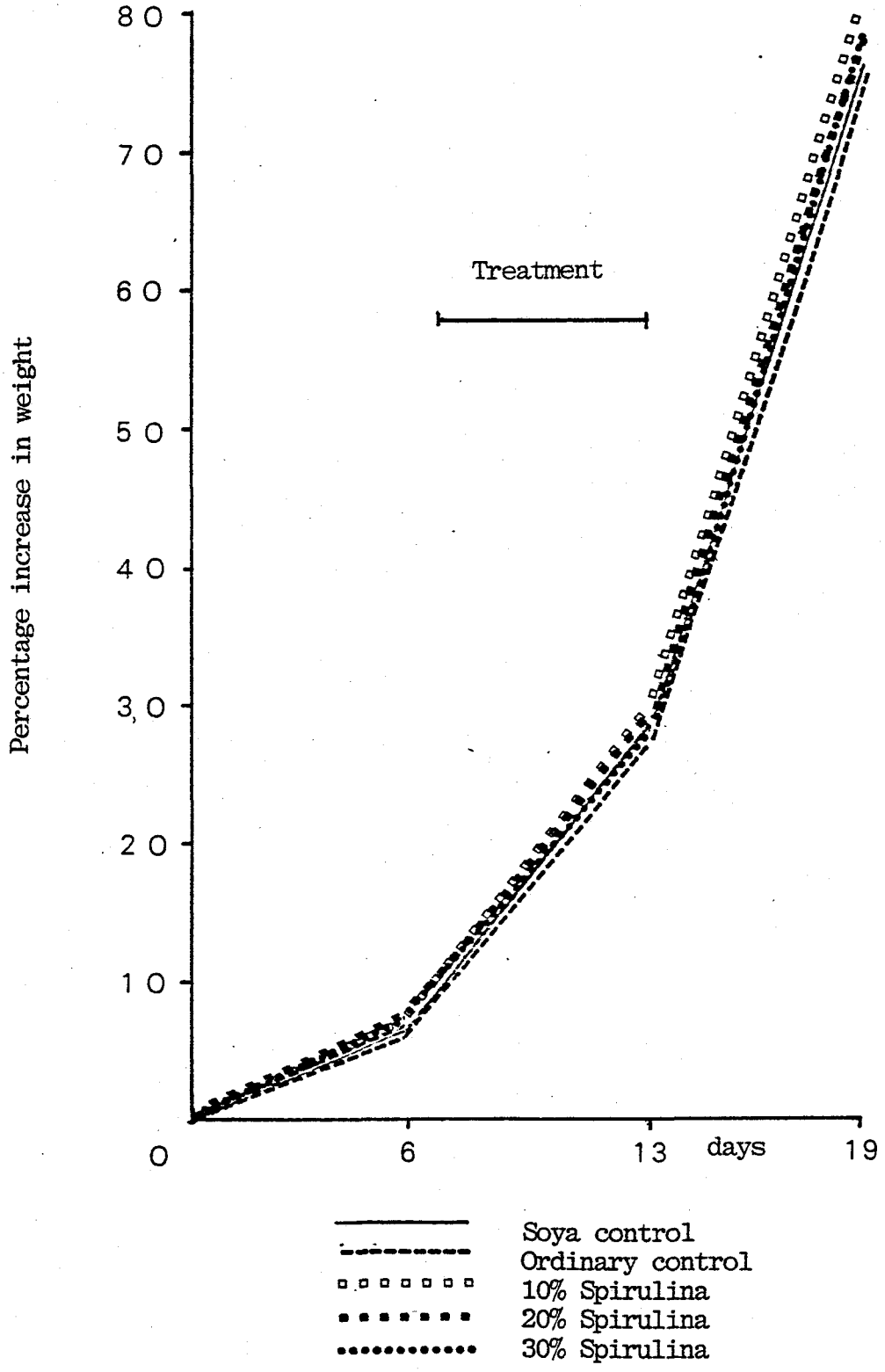


Fig.7 WEIGHT INCREASE IN MICE TREATED WITH SPIRULINA FROM THE 1st TO THE 13th DAY OF GESTATION

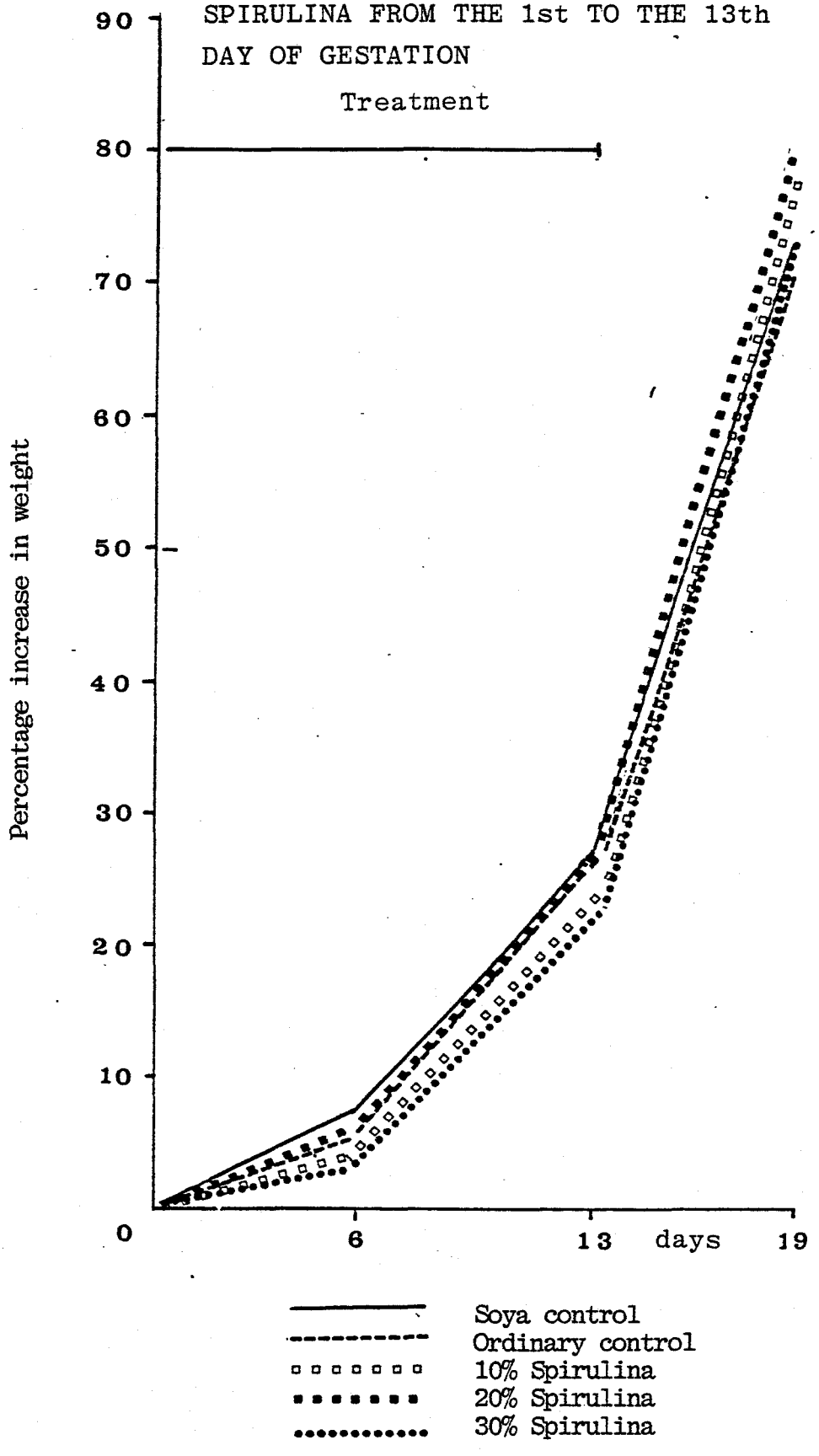
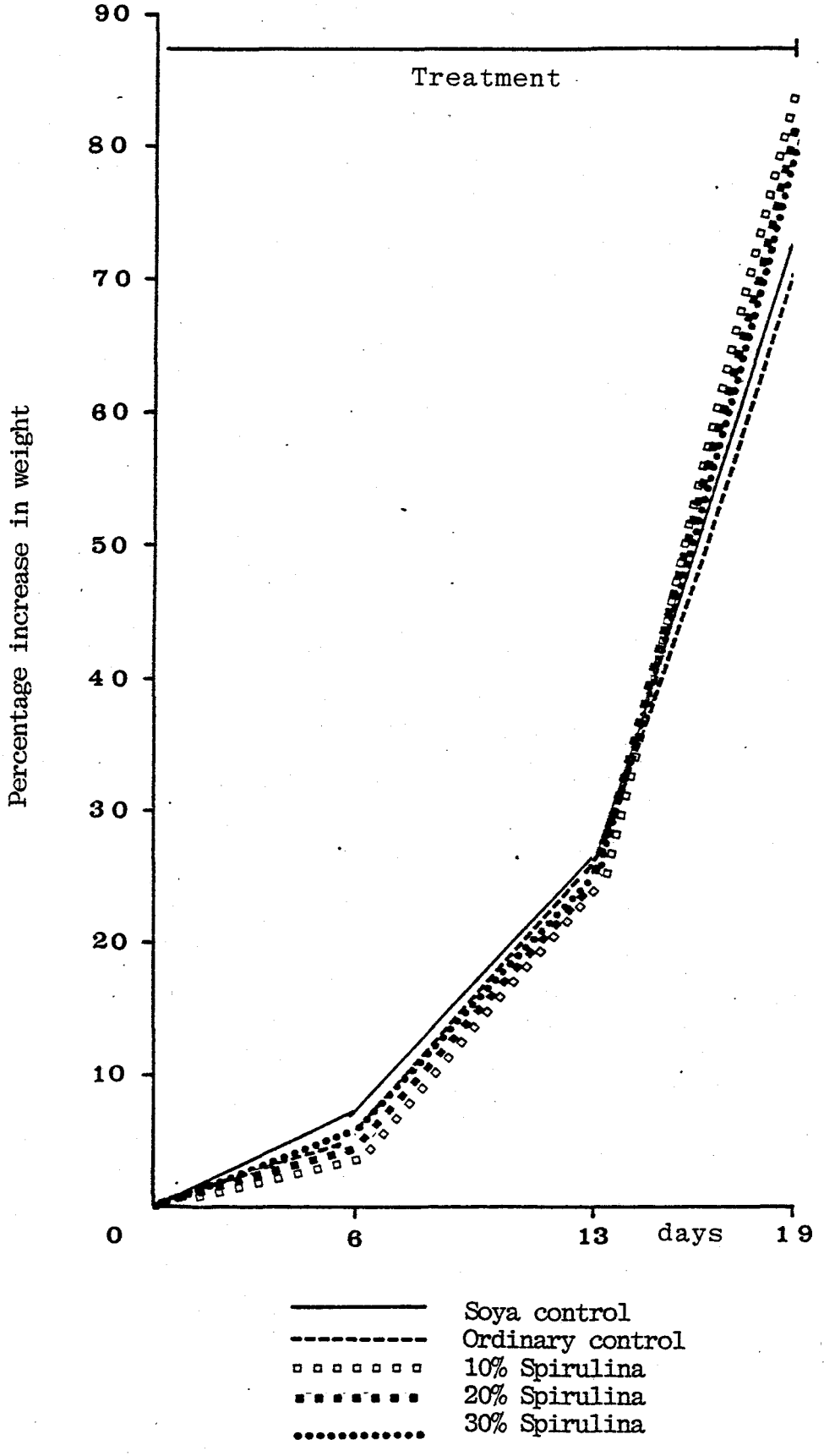


Fig.8 WEIGHT INCREASE IN MICE TREATED WITH SPIRULINA FROM THE 1st TO THE 19th DAY OF GESTATION



Teratogenic effect

Tables 25 to 32 again show the absolute data for the mothers and the foetuses, whilst the following tables show the percentages, the teratogenic indices and the descriptions of the abnormalities.

Table 33, relating to the mothers, shows that the frequency of those affected by the treatment with Spirulina is lower than the soya control. These mothers did, however, give rise to reabsorptions, unlike the control which shows an equal incidence of these with abnormal foetuses.

Similar results are found with the females fed during organogenèsis (Table 34), but in the case of the 30% Spirulina there is an equal frequency of mothers with reabsorbed and dead foetuses (2.2.1) as mothers with reabsorbed, dead or abnormal foetuses.

Table 35 shows also that the soya control gives a figure of 37.5 of mothers with affected litters; this corresponds with the experimental groups, but does not agree with the increase in the level of Spirulina. In the soya control group and the 10% Spirulina the incidents corresponding to mothers with reabsorptions and malformed foetuses are greater than for those mothers which show only reabsorptions.

Treatment of the mice from the 1st to the 19th day (Table 36) show that more affected mothers are produced than in the other periods of treatment; however this increase is characteristic of all groups, including the soya and ordinary controls.

In each case it will be noted that the majority of these females are due to the presence of reabsorptions or dead foetuses rising, with the highest level of Spirulina, to 33.3.

TABLE 25. EFFECT OF SPIRULINA ON MICE, INGESTED FROM THE 7th TO THE 9th DAY OF GESTATION

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of females:					
1. Mated	20	21	22	19	21
2. Fertile, with:	18	19	18	17	19
2.1. Normal litters	10	10	11	11	12
2.2. Litters affected by:	8	9	7	6	7
2.2.1. Reabsorbed or dead foetuses	4	4	5	4	4
2.2.2. Abnormal foetuses	3	4	1	1	1
2.2.3. Reabsorbed or dead and abnormal foetuses	1	1	1	1	2
3. Gestating at time of sacrifice	16	16	15	14	17

TABLE 26. EFFECT OF SPIRULINA ON MICE, INGESTED FROM THE 7th TO THE 13th DAY OF GESTATION

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of females:					
1. Mated	20	20	23	24	22
2. Fertile, with:	18	19	22	21	20
2.1. Normal litters	10	13	15	15	15
2.2. Litters affected by:	8	6	7	6	5
2.2.1. Reabsorbed or dead foetuses	4	4	5	3	2
2.2.2. Abnormal foetuses	3	1	0	2	1
2.2.3. Reabsorbed or dead or abnormal foetuses	1	1	2	1	2
3. Gestating at time of sacrifice	16	17	20	20	19

TABLE 27. EFFECT OF SPIRULINA ON MICE, INGESTED FROM THE 1st TO THE 13th DAY OF GESTATION

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of females:					
1. Mated	20	20	22	20	21
2. Fertile, with:	18	16	19	17	15
2.1. Normal litters	10	10	13	13	11
2.2. Litters affected by:	8	6	6	4	4
2.2.1. Reabsorbed or dead foetuses	4	2	1	2	1
2.2.2. Abnormal foetuses	3	1	1	0	2
2.2.3. Reabsorbed or dead or abnormal foetuses	1	3	4	2	1
3. Gestating at time of sacrifice	16	14	18	16	14

TABLE 28. EFFECT OF SPIRULINA ON MICE, INGESTED FROM THE 1st TO THE 19th DAY OF GESTATION

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of females:					
1. Mated	20	20	21	19	20
2. Fertile, with:	18	16	17	16	18
2.1. Normal litters	10	8	8	9	9
2.2. Litters affected by	8	8	9	7	9
2.2.1. Reabsorbed or dead foetuses	4	4	5	4	6
2.2.2. Abnormal foetuses	3	1	3	1	0
2.2.3. Reabsorbed or dead or abnormal foetuses	1	3	1	2	3
3. Gestating at time of sacrifice	16	14	15	14	15

TABLE 29. EFFECT OF SPIRULINA ON MICE, INGESTED FROM THE 7th TO THE 9th DAY OF GESTATION

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of:					
1. Implantations	182	192	202	169	190
2. Foetuses	138	146	162	124	159
2.1. Normal	133	141	157	121	153
2.2. Affected by:	49	51	45	48	37
2.2.1. Abnormalities	5	5	5	3	6
2.2.2. Reabsorption or death	44	46	40	45	31

TABLE 30. EFFECT OF SPIRULINA ON MICE, INGESTED FROM THE 7th TO THE 13th DAY OF GESTATION

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of:					
1. Implantations	182	189	229	223	206
2. Foetuses	138	152	184	188	175
2.1. Normal	133	146	181	183	171
2.2. Affected by:	49	43	48	40	35
2.2.1. Abnormalities	5	6	3	5	4
2.2.2. Reabsorption or death	44	37	45	35	31

TABLE 31. EFFECT OF SPIRULINA ON MICE, INGESTED FROM THE 1st TO THE 13th DAY OF GESTATION

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of:					
1. Implantations	182	156	181	179	130
2. Foetuses	138	119	158	147	124
2.1. Normal	133	115	152	145	120
2.2. Affected by:	49	41	29	34	18
2.2.1. Abnormalities	5	4	6	2	4
2.2.2. Reabsorption or death	44	37	23	32	14

TABLE 32. EFFECT OF SPIRULINA ON MICE, INGESTED FROM THE 1st TO THE 19th DAY OF GESTATION

"FOETUSES

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of:					
1. Implantations	182	159	165	159	164
2. Foetuses	138	113	123	118	124
2.1. Normal	133	110	116	113	108
2.2. Affected by:	49	49	49	46	56
2.2.1. Abnormalities	5	4	7	5	6
2.2.2. Reabsorption or death	44	45	42	41	50

TABLE 33. SPIRULINA IN MICE, INGESTED FROM THE 7th TO THE 9th DAY OF GESTATION. PERCENTAGES

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
% of females:					
2. Fertile, with:	90.0	90.5	81.8	89.5	90.5
2.1. Normal litters	55.5	52.6	61.1	64.7	63.1
2.2. Litters affected by:	44.4	47.3	38.9	35.3	36.8
2.2.1. Reabsorbed or dead foetuses	22.2	21.0	27.8	23.5	21.0
2.2.2. Abnormal foetuses	16.7	21.0	5.5	5.9	5.3
2.2.3. Reabsorbed or dead and abnormal foetuses	5.5	5.3	5.5	5.9	10.5
3. Gestating at time of sacrifice	88.9	84.2	83.3	82.3	89.5

TABLE 34. SPIRULINA IN MICE, INGESTED FROM THE 7th TO THE 13th DAY OF GESTATION. PERCENTAGES

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
% of females:					
2. Fertile, with:	90.0	95.0	95.6	87.5	90.9
2.1. Normal litters	55.5	68.4	68.2	71.4	75.0
2.2. Litters affected by:	44.4	31.6	31.8	28.6	25.0
2.2.1. Reabsorbed or dead foetuses	22.2	21.0	22.7	14,3	10,0
2.2.2. Abnormal foetuses	16.7	5.3	0.0	9.5	5.0
2.2.3. Reabsorbed or dead and abnormal foetuses	5.5	5.3	9.1	4.8	10.0
3. Gestating at time of sacrifice	88.9	89.5	90.9	95.2	95.0

TABLE 35. SPIRULINA IN MICE, INGESTED FROM THE 1st TO THE 13th DAY OF GESTATION. PERCENTAGES

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
% of females:					
2. Fertile, with:	90.0	80.0	86.4	85.0	71.4
2.1. Normal litters	55.5	62.5	68.4	76.5	73.3
2.2. Litters affected by:	44.4	37.5	31.6	23.5	26.7
2.2.1. Reabsorbed or dead foetuses	22.2	12.5	5.3	11.8	6.7
2.2.2. Abnormal foetuses	16.7	6.2	5.3	0.0	13.3
2.2.3. Reabsorbed or dead and abnormal foetuses	5.5	18.7	21.0	11.8	6.7
3. Gestating at time of sacrifice	88.9	87.5	94.7	94.0	93.3

TABLE 36. SPIRULINA IN MICE, INGESTED FROM THE 1st TO THE 19th DAY OF GESTATION. PERCENTAGES

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
% of females:					
2. Fertile, with:	90.0	80.0	80.9	84.2	90.0
2.1. Normal litters	55.5	50.0	47.0	56.2	50.0
2.2. Litters affected by:	44.4	50.0	52.9	43.7	50.0
2.2.1. Reabsorbed or dead foetuses	22.2	25.0	29.4	25.0	33.3
2.2.2. Abnormal foetuses	16.7	6.2	17.6	6.2	0.0
2.2.3. Reabsorbed or dead and abnormal foetuses	5.5	18.7	5.9	12.5	16.7
3. Gestating at time of sacrifice	88.9	87.5	88.2	87.5	83.3

In all the tables referring to the foetuses (Tables 37 to 40) similar frequencies of incidents are observed in the soya control groups and the Spirulina groups. In the same way reabsorbed or dead foetuses represent the majority of such incidents in all the periods of treatment.

Tables 37 and 38 do, however, indicate some significant differences in the mean weight of the foetuses and in the mean number of foetuses for each pregnant female.

TABLE 37. SPIRULINA IN MICE, INGESTED FROM THE 7th to the 9th DAY OF GESTATION. PERCENTAGES

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
% of foetuses:					
2.1. Normal	73.1	75.7	77.7	71.6	80.5
2.2. Affected by:	26.9	26.5	22.3	28.4	19.5
2.2.1. Abnormalities	2.7	2.6	2.5	1.8	3.1
2.2.2. Reabsorptions or death	24.2	23.9	19.8	26.6	16.3
Mean of:					
- foetal weight (g)	1.40	1.33	1.38	1.43	1.36
- implantations per fertile female	10.1	10.1	11.2*	9.9	10.0
- foetuses per pregnant female	8.6*	10.2	10.8	8.8	9.3

TABLE 38. SPIRULINA IN MICE, INGESTED FROM THE 7th TO THE 13th DAY OF GESTATION. PERCENTAGES

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	% Spirulina 20	% Spirulina 30
% of foetuses:					
2.1. Normal	73.1	77.2	79.0	82.0	83.0
2.2. Affected by	26.9	22.7	21.0	17.9	17.0
2.2.1. Abnormalities	2.7	3.2	1.3	2.2	1.9
2.2.2. Reabsorptions or death	24.2	19.6	19.6	15.7	15.0
Mean of:					
- foetal weight (g)	1.40	1.33	1.38	1.30	1.36
- implantations per fertile female	10.1	9.9	10.4	10.6	10.3
- foetuses per pregnant female	8.6*	8.9	9.2	9.9*	9.7

TABLE 39. SPIRULINA IN MICE, INGESTED FROM THE 1st TO THE 13th DAY OF GESTATION. PERCENTAGES

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
% of foetuses:					
2.1. Normal	73.1	73.7	84.0	81.0	86.9
2.2. Affected by:	26.9	26.3	16.0	19.0	13.0
2.2.1. Abnormalities	2.7	2.6	3.3	1.1	2.9
2.2.2. Reabsorptions or death	24.2	23.7	12.7	17.9	10.1
Mean of:					
- foetal weight (g)	1.40	1.37	1.41	1.36	1.42
- implantations per fertile female	10.1	9.7	9.5	10.5	9.2
- foetuses per pregnant female	8.6*	8.5	8.7	9.1	8.8

TABLE 40. SPIRULINA IN MICE, INGESTED FROM THE 1st TO THE 19th DAY OF GESTATION. PERCENTAGES

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
% of foetuses:					
2.1. Normal	73.1	69.2	70.3	71.1	65.8
2.2. Affected by:	26.9	30.8	29.7	28.9	34.1
2.2.1. Abnormalities	2.7	2.5	4.2	3.1	3.6
2.2.2. Reabsorptions or death	24.2	28.3	25.4	25.8	30.5
Mean of:					
- foetal weight (g)	1.40	1.34	1.36	1.40	1.38
- implantations per fertile female	10.1	9.9	9.7	9.9	9.1
- foetuses per pregnant female	8.6*	8.1	8.2	8.4	8.2

Based on the previous data the following tables (Tables 41 to 44) show that the teratogenic index is negative in practically all cases, showing however low values such as -22.8 in the case of ingestion from the 6th to the 8th day (Table 41); -23 with treatment from the 7th to the 13th day and -22.4 from the 1st to the 13th day. These indices are lower than those previously encountered with the rats, and show that Spirulina does not have any teratogenic effects.

TABLE 41. SPIRULINA IN MICE, INGESTED FROM THE 7th TO THE 9th DAY OF GESTATION. TERATOGENIC INDICES

% Spirulina	In the mothers	In the foetuses
10	- 16.0	- 2.6
20	- 22.8	5.4
30	- 20.0	- 6.3
Soya control	5.2	- 3.6

TABLE 42. SPIRULINA IN MICE, INGESTED FROM THE 7th TO THE 13th DAY OF GESTATION. TERATOGENIC INDICES

% Spirulina	In the mothers	In the foetuses
10	- 0.3	- 2.2
20	- 4.4	- 6.2
30	- 9.6	- 7.3
Soya control	- 23.0	- 5.7

TABLE 43. SPIRULINA IN MICE, INGESTED FROM THE 1st TO THE 13th DAY OF GESTATION. TERATOGENIC INDICES

% Spirulina	In the mothers	In the foetuses
10	- 9.4	- 14.0
20	- 22.4	- 9.9
30	- 17.3	- 18.0
Soya control	- 12.4	0.8

TABLE 44. SPIRULINA IN MICE, INGESTED FROM THE 1st TO THE 19th DAY OF GESTATION. TERATOGENIC INDICES

% Spirulina	In the mothers	In the foetuses
10	5.8	- 1.6
20	- 12.6	- 2.7
30	0.0	4.8
Soya control	10.1	- 5.3

The type of abnormalities encountered in the mice are listed in the following tables (Tables 45 to 48).

External examination of the foetuses produced by mothers fed on the diets from the 7th to the 9th day of gestation (Table 45) indicates that the anomalies are qualitatively very comparable to those found in the rats. These include macerated foetuses and foetuses with haematomas. In the same way examination of the skeletons indicates cases of defects in the ribs and faults in ossification, whilst the Wilson sections showed malformations such as hydronephrosis. One isolated case of split palate was however observed with 30% of Spirulina.

Table 46 shows a case of exencephalitis with 10% Spirulina. Also noted were defects in the ribs, poor ossification and defects in the vertebrae, and these were observed in both the treated and control groups.

Table 47 lists the malformations or anomalies which were found; these were distributed irregularly over all the groups. The same can be observed in table 48 in which an isolated case of exencephalitis can be seen with 10% Spirulina. Other minor malformations were detected using the Wilson technique.

TABLE 45. DESCRIPTION OF THE ABNORMAL FOETUSES PRODUCED BY MICE TREATED WITH SPIRULINA FROM THE 7th TO THE 9th DAY OF GESTATION

Type of examination	Ordinary control	Soya control	% Spirulina 10	% Spirulina 20	% Spirulina 30
EXTERNAL	1f. macerated 1f. haematoma	1f. macerated 1f. exencephalus 1f. haematoma 1f. faulty development	2f. macerated	1f. generalized oedema	2f. haematoma
+	138	164	162	124	159
ALIZARINE	1f. faulty craneal ossification		2f. fused ribs	1f. general retarded ossifn.	2f. reduction of 13th ribs 1f. general retarded ossifn.
+	80	108	110	81	101
WILSON	1f. pulmonary hypoplasia 1f. subcutaneous oedema	1f. unilateral hydronephrosis	1f. renal ectopia	1f. bilateral hydronephrosis	1f. cleft palate
+	58	56	52	43	58

+ Number of foetuses examined.

TABLE 46. DESCRIPTION OF THE ABNORMAL FOETUSES PRODUCED BY MICE TREATED WITH SPIRULINA FROM THE 7th TO THE 13th DAY OF GESTATION

Type of examination	Ordinary control	Soya control	% Spirulina 10	20	30
EXTERNAL	1f. macerated 1f. haematoma	3f. haematomas	1f* exencephalus	2f. generalized oedema	
+	138	152	216	202	185
ALIZARIN	1f. faulty craneal ossification	2f. malformed ribs 1f. general failure of ossifn.	1f. poor craneal ossification 1f* fused ribs	2f. without presacral vertebra 1f. general failure of ossifn.	2f. malformed ribs 2f. general failure of ossifn.
+	80	93	142	138	121
WILSON	1f. pulmonary hypoplasia 1f. subcutaneous oedema		1f. bilateral hydrocephalus		1f. subcutaneous oedema
+	58	59	74	64	64

\+ Number of foetuses examined.

* Same foetus.

TABLE 47. DESCRIPTION OF THE ABNORMAL FOETUSES PRODUCED BY MICE TREATED WITH SPIRULINA FROM THE 1st TO THE 13th DAY OF GESTATION

Type of examination	Ordinary control	Soya control	% Spirulina 10	20	30
EXTERNAL	1f. macerated 1f. haematoma	2f. generalized oedema 1f. haematoma	3f. macerated 1f. faulty development	1f. macerated 1f. faulty development	2f. haematomas
+	138	119	158	147	124
ALIZARINE	1f. faulty craneal ossification				
+	80	82	99	98	82
WILSON	1f. pulmonary hypoplasia 1f. subcutaneous oedema	1f. intracraneal haemorrhage	1f. intrabdominal haemorrhage 1f. pulmonary hypoplasia		1f. unilateral hydronephrosis 1f. renal ectopia
+	58	37	59	49	42

+ Number of foetuses examined.

TABLE 48. DESCRIPTION OF THE ABNORMAL FOETUSES PRODUCED BY MICE TREATED WITH SPIRULINA FROM THE 1st TO THE 19th DAY OF GESTATION

Type of examination	Ordinary control	Soya control	% Spirulina 10	20	30
EXTERNAL	1f. macerated		2f. macerated		2f. macerated
			1f. exencephalus		
	1f. haematoma		1f. generalized oedema	2f. generalized oedema	
+	138	114	123	118	114
ALIZARINE	1f. faulty craneal ossification	1f. fused ribs	1f. retarded craneal ossification	1f. general retarded ossification	
		1f. extra presacral vertebra			1f. large cervical rib
+	80	79	75	81	81
WILSON	1f. pulmonary hypoplasia	1f. intracraneal haemorrhage	2f. pulmonary hypertrophia	1f. intrabdominal haemorrhage	2f. unilateral hydrocephalus
	1f. subcutaneous oedema	1f. enlarged kidney		1f. subcutaneous oedema	
+	58	35	48	37	33

+ Number of foetuses examined.

HAMSTERS

Increase in weight

The percentage weight increase of females fed from the 7th to the 9th day of gestation with the various diets containing Spirulina shows no change, as can be seen from figure 9.

Figures 10, 11 and 12, corresponding to the other periods of treatment, show considerable differences when compared with the soya control.

In general it can be seen that changes in the rate of growth of hamsters fed on the experimental and control diets is less than with the rat and mouse.

Fig.9 WEIGHT INCREASE IN HAMSTERS TREATED WITH SPIRULINA FROM THE 7th TO THE 9th DAY OF GESTATION

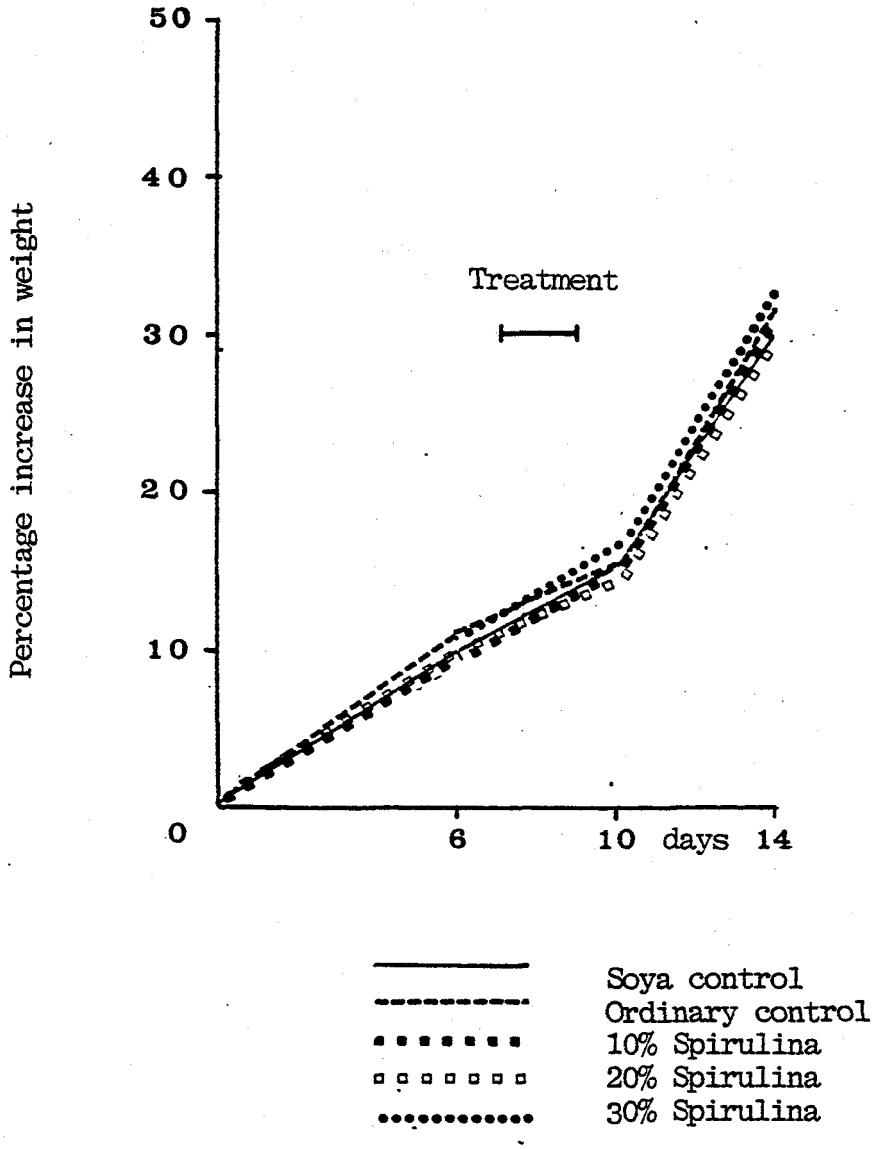


Fig.10 WEIGHT INCREASE IN HAMSTERS TREATED WITH SPIRULINA FROM THE 7th TO THE 11th DAY OF GESTATION

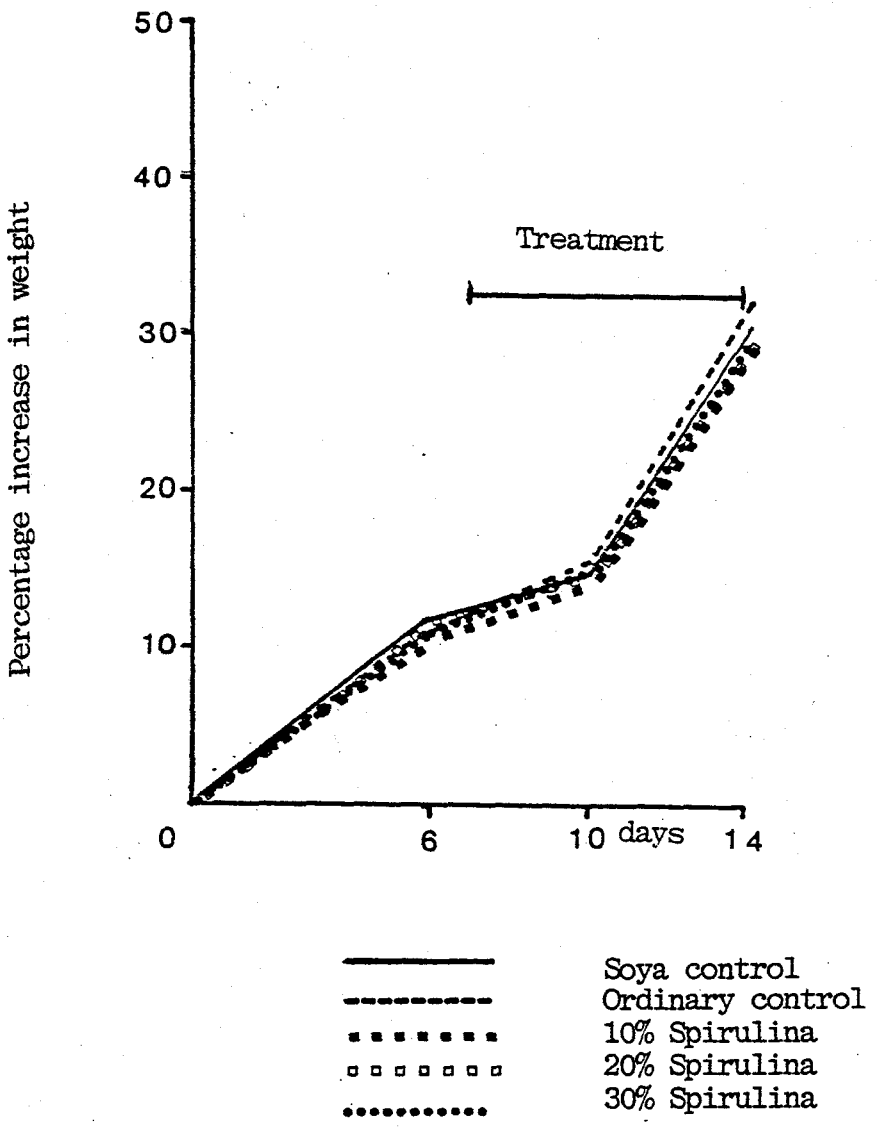


Fig.11 WEIGHT INCREASE IN HAMSTERS TREATED WITH SPIRULINA FROM THE 1st TO THE 11th DAY OF GESTATION

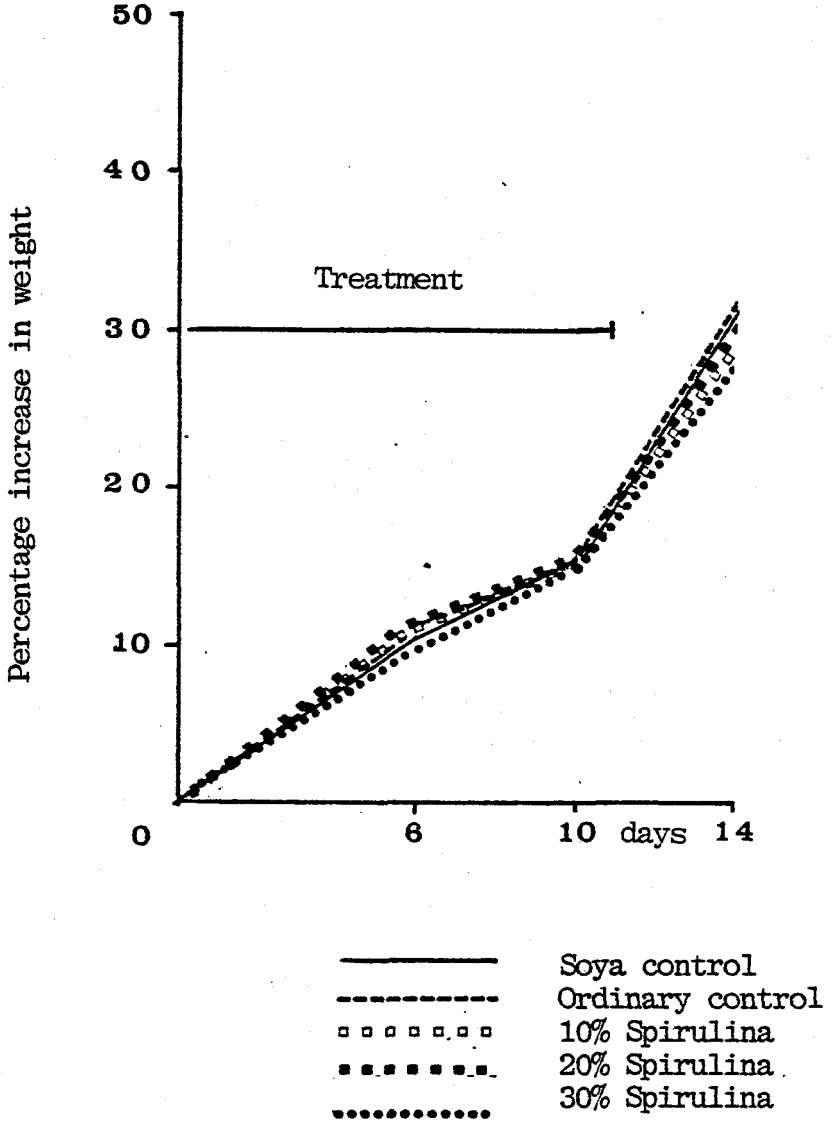
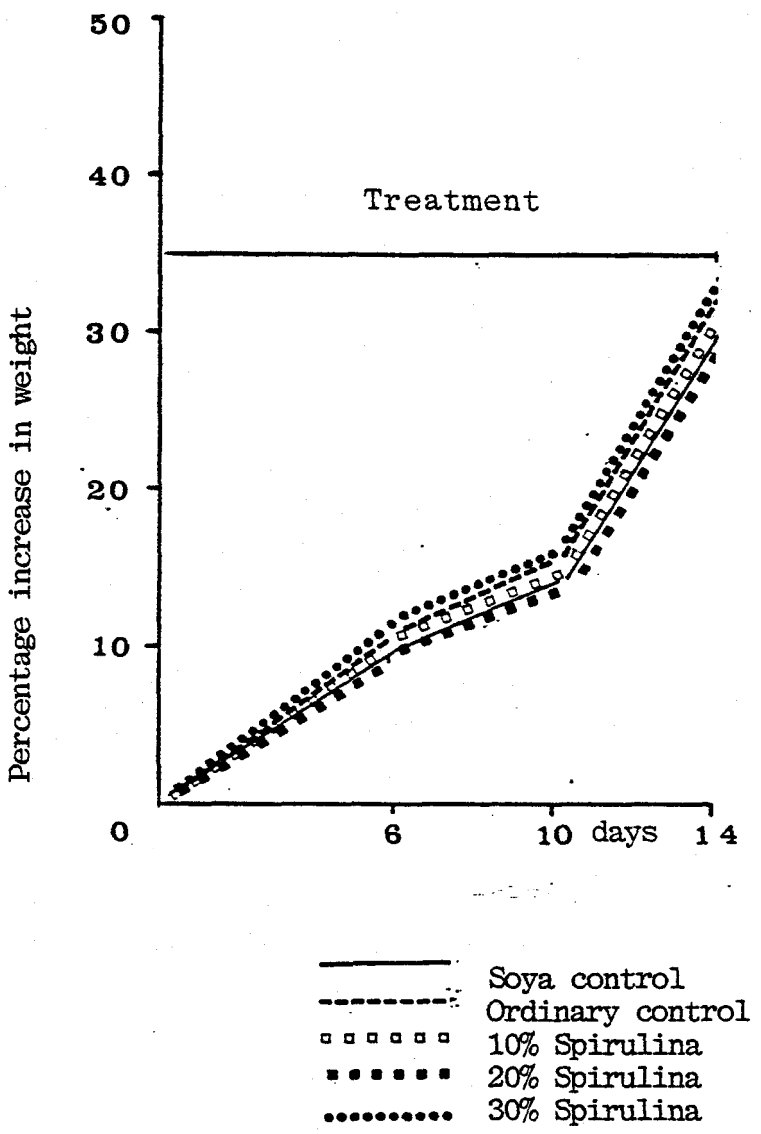


Fig.12 WEIGHT INCREASE IN HAMSTERS TREATED WITH SPIRULINA FROM THE 1st TO THE 14th DAY OF GESTATION



Teratogenic effect

Tables 49 to 56 show the absolute values, and the following tables the percentages, indices and description of the abnormalities found, as in the case of the rats and mice.

Table 57 shows the results of the ingestion of Spirulina from the 7th to the 9th day of gestation. It can be observed that the affected litters do not show any differences with respect to the control and include mothers with reabsorbed or dead foetuses and mothers showing both reabsorbed or dead and abnormal foetuses. Mothers with abnormal foetuses indicate a value of 0 for the soya control as compared with 10 and 30% Spirulina.

Table 58 indicates that the values corresponding to 10 and 30% Spirulina show a reduction with respect to the soya control. In this control, and with a level of 20% Spirulina, the incidents are mainly due to mothers with reabsorbed or dead foetuses.

The results shown in table 59 do not indicate any differences against the controls. It is notable that the concentration of 30% gives rise mainly to mothers with embryonic reabsorption, whilst none of the treatments with Spirulina result in mothers that only show foetuses with abnormalities. They are always accompanied by reabsorptions.

The overall effect of the incidents is not different between the control and treated groups in Table 60. In the soya control many of the affected litters are due to the mothers which only show reabsorbed foetuses; to a lesser extent this is shown at 30%. At levels of 10 and 20% the results are the opposite; here it is females with reabsorbed and abnormal foetuses which form the majority of the incidents.

TABLE 49. THE EFFECT OF SPIRULINA ON HAMSTERS, INGESTED FROM THE 7th TO THE 9th DAY OF GESTATION

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of females:					
1. Mated	20	19	19	18	21
2. Fertile, with:	18	17	17	17	19
2.1. Normal litters	14	14	13	13	15
2.2. Litters affected by:	4	3	4	4	4
2.2.1. Reabsorbed or dead foetuses	1	1	2	2	2
2.2.2. Abnormal foetuses	1	0	0	1	0
2.2.3. Reabsorbed or dead and abnormal foetuses	2	2	2	1	2
3. Gestating at sacrifice	18	16	17	16	18

TABLE 50. THE EFFECT OF SPIRULINA ON HAMSTERS, INGESTED FROM THE 7th TO THE 11th DAY OF GESTATION

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of females:					
1. Mated	20	19	22	20	18
2. Fertile, with:	18	18	19	17	17
2.1. Normal litters	14	12	14	11	14
2.2. Litters affected by:	4	6	5	6	3
2.2.1. Reabsorbed or dead foetuses	1	3	2	4	1
2.2.2. Abnormal foetuses	1	1	1	0	1
2.2.3. Reabsorbed or dead and abnormal foetuses	2	2	2	2	1
3. Gestating at sacrifice	18	17	17	16	16

TABLE 51. THE EFFECT OF SPIRULINA ON HAMSTERS, INGESTED FROM THE 1st TO THE 11th DAY OF GESTATION

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of females:					
1. Mated	20	19	21	18	17
2. Fertile, with:	18	18	20	18	17
2.1. Normal litters	14	13	16	14	12
2.2. Litters affected by:	4	5	4	4	5
2.2.1. Reabsorbed or dead foetuses	1	2	1	2	4
2.2.2. Abnormal foetuses	1	1	0	0	0
2.2.3. Reabsorbed or dead and abnormal foetuses	2	2	3	2	1
3. Gestating at sacrifice	18	17	19	17	16

TABLE 52. THE EFFECT OF SPIRULINA ON HAMSTERS, INGESTED FROM THE 1st TO THE 14th DAY OF GESTATION

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of females:					
1. Mated	20	22	18	20	21
2. Fertile, with:	18	21	16	19	9
2.1. Normal litters	14	16	13	15	14
2.2. Litters affected by:	4	5	3	4	5
2.2.1. Reabsorbed or dead foetuses	1	4	1	1	3
2.2.2. Abnormal foetuses	1	1	0	1	0
2.2.3. Reabsorbed or dead and abnormal foetuses	2	0	2	2	2
3. Gestating at sacrifice	18	19	15	18	19

TABLE 53. THE EFFECT OF SPIRULINA ON HAMSTERS, INGESTED FROM THE 7th TO THE 9th DAY OF GESTATION

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of:					
1. Implantations	207	189	204	201	217
2. Foetuses	197	173	182	178	198
2.1. Normal	191	170	178	173	196
2.2. Affected by:	16	19	26	28	21
2.2.1. Abnormalities	6	3	4	5	2
2.2.2. Reabsorption or death	10	16	22	23	19

TABLE 54. THE EFFECT OF SPIRULINA ON HAMSTERS, INGESTED FROM THE 7th TO THE 11th DAY OF GESTATION

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of:					
1. Implantations	207	202	204	198	189
2. Foetuses	197	181	177	173	170
2.1. Normal	191	176	173	169	167
2.2. Affected by:	16	26	31	29	22
2.2.1. Abnormalities	6	5	4	4	3
2.2.2. Reabsorption or death	10	21	27	25	19

TABLE 55. THE EFFECT OF SPIRULINA ON HAMSTERS, INGESTED FROM THE 1st TO THE 11th DAY OF GESTATION

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of:					
1. Implantations	207	204	242	197	187
2. Foetuses	197	187	227	176	168
2.1. Normal	191	182	220	172	166
2.2. Affected by:	16	22	22	25	21
2.2.1. Abnormalities	6	5	7	4	2
2.2.2. Reabsorption or death	10	17	15	21	19

TABLE 56. THE EFFECT OF SPIRULINA ON HAMSTERS, INGESTED FROM THE 1st TO THE 14th DAY OF GESTATION

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Number of:					
1. Implantations	207	227	178	221	215
2. Foetuses	197	198	159	193	200
2.1. Normal	191	194	156	190	195
2.2. Affected by:	16	33	22	31	20
2.2.1. Abnormalities	6	4	3	3	5
2.2.2. Reabsorption or death	10	29	19	28	15

TABLE 57. SPIRULINA IN HAMSTERS, INGESTED FROM THE 7th TO THE 9th DAY OF GESTATION. PERCENTAGES

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Percentage of females:					
2. Fertile, with:	90.0	89.5	89.5	94.4	90.5
2.1. Normal litters	77.8	82.3	76.5	76.5	78.9
2.2. Litters affected by:	22.2	17.6	23.5	23.5	21.0
2.2.1. Reabsorbed or dead foetuses	5.5	5.9	11.7	11.8	10.5
2.2.2. Abnormal foetuses	5.5	0.0	0.0	5.8	0.0
2.2.3. Reabsorbed or dead and abnormal foetuses	11.1	11.7	11.7	5.8	10.5
3. Gestating at sacrifice	100.0	94.1	100.0	94.1	94.7

TABLE 58. SPIRULINA IN HAMSTERS, INGESTED FROM THE 7th TO THE 11th DAY OF GESTATION. PERCENTAGES

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Percentage of females:					
2. Fertile, with:	90.0	94.7	86.4	85.0	94.0
2.1. Normal litters	77.8	66.7	73.7	64.7	82.3
2.2. Litters affected by:	22.2	33.3	26.3	35.3	17.7
2.2.1. Reabsorbed or dead foetuses	5.5	16.7	10.5	23.5	5.9
2.2.2. Abnormal foetuses	5.5	5.5	5.3	0.0	5.9
2.2.3. Reabsorbed or dead and abnormal foetuses	11.1	11.1	10.5	11.8	5.9
3. Gestating at sacrifice	100.0	94.4	89.5	94.1	94.1

TABLE 59. SPIRULINA IN HAMSTERS, INGESTED FROM THE 1st TO THE 11th DAY OF GESTATION. PERCENTAGES

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Percentage of females:					
2. Fertile, with:	90.0	94.7	95.2	100.0	100.0
2.1. Normal litters	77.8	72.2	80.0	77.8	70.6
2.2. Litters affected by:	22.2	27.8	20.0	22.2	29.4
2.2.1. Reabsorbed or dead foetuses	5.5	11.1	5.0	11.1	23.5
2.2.2. Abnormal foetuses	5.5	5.5	0.0	0.0	0.0
2.2.3. Reabsorbed or dead and abnormal foetuses	11.1	11.1	15.0	11.1	5.9
3. Gestating at sacrifice	100.0	94.4	95.0	94.4	94.1

TABLE 60. SPIRULINA IN HAMSTERS, INGESTED FROM THE 1st TO THE 14th DAY OF GESTATION. PERCENTAGES

"MOTHERS"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Percentage of females:					
2. Fertile, with:	90.0	95.4	88.9	95.0	90.5
2.1. Normal litters	77.8	76.2	81.2	78.9	73.7
2.2. Litters affected by:	22.2	23.8	18.8	21.0	26.3
2.2.1. Reabsorbed or dead foetuses	5.5	19.0	6.2	5.2	15.8
2.2.2. Abnormal foetuses	5.5	4.8	0.0	5.2	0.0
2.2.3. Reabsorbed or dead and abnormal foetuses	11.1	0.0	12.5	10.5	10.5
3. Gestating at sacrifice	100.0	90.5	93.7	94.7	100.0

The results for the foetuses, which are shown in table 61, indicate that there is no apparent difference in the incidence of affected foetuses in relation to the control.

As in the previous cases embryonic reabsorptions are the principal causes of the incidents.

This is, however, the case with foetuses produced by mothers treated during organogenesis, as is shown in table 62, and in the case of the other periods of treatment as can be seen from the subsequent tables (Tables 63 and 64).

In regard to the mean foetal weight and the number of foetuses per pregnant female, there are significant differences with 20 and 10% of Spirulina respectively.

TABLE 61. SPIRULINA IN HAMSTERS, INGESTED FROM THE 7th TO THE 9th DAY OF GESTATION. PERCENTAGES

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Percentage of foetuses:					
2.1. Normal	92.3	89.9	87.2	86.1	90.3
2.2. Affected by:	7.7	10.0	12.7	13.9	9.7
2.2.1. Abnormalities	2.9	1.6	1.9	2.5	0.9
2.2.2. Reabsorption or death	4.8	8.4	10.8	11.4	8.7
Mean of:					
- foetal weight (g)	1.57	1.62	1.64	1.56	1.60
- implantations/fertile female	11.5	11.1	12.0	11.8	11.4
- foetuses/pregnant female	10.9	10.8	10.7	11.1	11.0

TABLE 62. SPIRULINA IN HAMSTERS, INGESTED FROM THE 7th TO THE 11th DAY OF GESTATION. PERCENTAGES

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Percentage of foetuses:					
2.1. Normal	92.3	87.1	84.8	85.3	88.3
2.2. Affected by:	7.7	12.9	15.2	14.6	11.6
2.2.1. Abnormalities	2.9	2.5	2.0	2.0	1.6
2.2.2. Reabsorption or death	4.8	10.4	13.2	12.6	10.0
Mean of:					
- foetal weight (g)	1.57	1.60	1.53	1.64	1.55
- implantations/fertile female	11.5	11.2	10.7	11.6	11.1
- foetuses/pregnant female	10.9	10.6	10.4	10.8	10.6

TABLE 63. SPIRULINA IN HAMSTERS, INGESTED FROM THE 1st TO THE 11th DAY OF GESTATION. PERCENTAGES

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Percentage of foetuses:					
2.1. Normal	92.3	89.2	90.9	87.3	88.8
2.2. Affected by:	7.7	10.8	9.1	12.7	11.2
2.2.1. Abnormalities	2.9	2.4	2.9	2.0	1.0
2.2.2. Reabsorption or death	4.8	8.3	6.2	10.6	10.2
Mean of:					
- foetal weight (g)	1.57	1.59	1.53	1.51*	1.60
- implantations/fertile female	11.5	11.3	12.1	10.9	11.0
- foetuses/pregnant female	10.9	11.0	11.9*	10.3	10.5

TABLE 64. SPIRULINA IN HAMSTERS, INGESTED FROM THE 1st TO THE 14th DAY OF GESTATION. PERCENTAGES

"FOETUSES"

Diet	Ordinary control	Soya control	% Spirulina 10	20	30
Percentage of foetuses:					
2.1. Normal	92.3	85.5	87.6	86.0	90.7
2.2. Affected by:	7.7	14.5	12.3	14.0	9.3
2.2.1. Abnormalities	2.9	1.7	1.7	1.3	2.3
2.2.2. Reabsorption or death	4.8	12.8	10.6	12.7	7.0
Mean of:					
- foetal weight (g)	1.57	1.52	1.49	1.56	1.58
- implantations/fertile female	11.5	10.8	11.1	11.6	11.3
- foetuses/pregnant female	10.9	10.4	10.6	10.7	10.5

The indices calculated in tables 65 to 68 show that, once again, Spirulina does not produce any teratogenic effects, whether considered in the light of mothers which give rise to affected foetuses or to the affected foetuses themselves.

TABLE 65. SPIRULINA IN HAMSTERS, INGESTED FROM THE 7th TO THE 9th DAY OF GESTATION. TERATOGENIC INDICES

% Spirulina	In the mothers	In the foetuses
10	7.1	3.0
20	7.1	4.3
30	4.1	0.3
Soya control	- 5.9	2.5

TABLE 66. SPIRULINA IN HAMSTERS, INGESTED FROM THE 7th TO THE 11th DAY OF GESTATION. TERATOGENIC INDICES

% Spirulina	In the mothers	In the foetuses
10	- 10.5	2.6
20	- 0.3	1.9
30	- 23.4	- 1.5
Soya control	14.3	5.6

TABLE 67. SPIRULINA IN HAMSTERS, INGESTED FROM THE 1st TO THE 11th DAY OF GESTATION. TERATOGENIC INDICES

% Spirulina	In the mothers	In the foetuses
10	- 10.8	- 1.9
20	- 7.7	2.1
30	2.2	0.4
Soya control	7.2	3.3

TABLE 68. SPIRULINA IN HAMSTERS, INGESTED FROM THE 1st TO THE 14th DAY OF GESTATION. TERATOGENIC INDICES

% Spirulina	In the mothers	In the foetuses
10	- 6.6	- 2.6
20	- 3.7	- 0.6
30	3.3	- 6.1
Soya control	2.0	7.4

The malformations and anomalies found in the hamsters in the control group were repeated on many occasions in the treated groups.

This is the case with the haemorrhages observed in the external examination, and also in the Wilson serial sections in table 69. In the skeleton the anomalies principally affected the sternum in the case of the control and the ribs in the experimental groups.

Table 70 shows similar anomalies. Particular malformations which were not encountered in the control group included one case of celosomia and two of bilateral hydrocephalitis, but similar cases were also found in the rat and mouse in both the control and treated groups.

As can be observed from table 71 there is an absence of external anomalies at levels of 20 and 30% Spirulina ingested from the 1st to the 11th day. Skeletal anomalies are, however, common to all groups, involving the cranium, sternum, pubis and ribs.

Finally table 72 shows some sporadic cases of malformations and anomalies, encountered in numerous cases divided amongst all the groups; these therefore exclude Spirulina as a possible cause.

TABLE 69. DESCRIPTION OF THE ABNORMAL FOETUSES PRODUCED BY HAMSTERS TREATED WITH SPIRULINA FROM THE 7th TO THE 9th DAY OF GESTATION

Type of examination	Ordinary control	Soya control	% Spirulina 10	20	30
EXTERNAL	1f. haema-tomas	1f. haemor-rage	1f. haema-toma	1f. exen-cephalus	1f* haemor-rage
+	197	173	182	178	198
ALIZARINE	1f. poor craneal ossification		1f. poor craneal ossification	2f. mal-formed ribs	
	1f. incomplete sternum	1f. bi-partite sternum	1f. fused ribs	1f. extra ribs	1f* extra ribs
+	131	118	119	122	134
WILSON	1f. enlarged renal pelvis	1f. internal haemorrhage	1f. hydro-cephalus	1f. internal haemorrhage	
+	66	55	63	56	64

\+ Number of foetuses examined

* Same foetus.

TABLE 70. DESCRIPTION OF THE ABNORMAL FOETUSES PRODUCED BY HAMSTERS TREATED WITH SPIRULINA FROM THE 7th TO THE 11th DAY OF GESTATION

Type of examination	Ordinary control	Soya control	% Spirulina 10	20	30
EXTERNAL	1f. haema-toma	1f* general-ized oedema		1f. celo-somia	1f. haema-toma
+	197	181	177	173	170
ALIZARINE	3f. poor craneal ossification	2f. general retarded ossification	2f. general retarded ossification	1f. fused ribs	2f. mal-formed ribs
	1f. incomplete sternum	1f. extra ribs	2f. poor craneal ossification	1f. mal-formed ribs	
		1f. malformed ribs			
+	131	119	122	113	114
WILSON	1f. enlarged renal pelvis	1f* renal ectopia		1f. bi-lateral hydro-cephalus	1f. bi-lateral hydro-cephalus
+	66	62	55	60	56

\+ Number of foetuses examined

* Same foetus

TABLE 71. DESCRIPTION OF THE ABNORMAL FOETUSES PRODUCED BY HAMSTERS TREATED WITH SPIRULINA FROM THE 1st TO THE 11th DAY OF GESTATION

Type of examination	Ordinary control	Soya control	% Spirulina 10	20	30
EXTERNAL	1f. haema-tomas	1f. general-ized oedema	2f. haema-tomas		
+	197	187	227	176	168
ALIZARINE	3f. poor craneal ossification	1f. fused ribs	2f. fused ribs	1f. 13th rib not developed	1f. sternum incompletely ossified
	1f. sternum incomplete	2f. poor ossification of pubis	2f. extra ribs	2f. poor craneal ossificn.	1f. poor ossification of pubis
+	129	128	156	112	112
WILSON	1f. enlarged renal pelvis	1f. hydro-cephalus	1f. renal ectopia	1f. uni-lateral hydro-cephalus	
+	68	59	71	64	56

+ Number of foetuses examined.

TABLE 72. DESCRIPTION OF THE ABNORMAL FOETUSES PRODUCED BY HAMSTERS TREATED WITH SPIRULINA FROM THE 1st TO THE 14th DAY OF GESTATION

Type of examination	Ordinary control	Soya control	% Spirulina 10	20	30
EXTERNAL	1f. haema-tomas	1f* haema-toma			1f. general-ized oedema
+	197	198	159	193	200
ALIZARINE	1f. poor craneal ossification 1f. sternum incomplete	2f. bi-partite sternum 1f* poor centro-vertebral ossificn.	2f. extra ribs	1f. bi-partite sternum 1f. poor craneal ossificn.	2f. fused ribs 2f. poor centro-vertebral ossificn.
+	129	131	109	131	130
WILSON	1f. enlarged renal pelvis	1f. internal oedema	1f. uni-lateral hydrocephalus	1f. internal oedema	
+	68	67	50	62	70

\+ Number of foetuses examined

* Same foetus.

DISCUSSION

Amongst the teratogenic studies which have been recommended by international organizations for unicellular proteins in the food products sector the only studies which have been reported in the literature refer to proteins of a different origin from that of algae.

However the same safety rules apply when carrying out tests on sub-acute and chronic toxicity, functional tests, etc., and these have made it possible to carry out tests on teratogenicity without exaggerating the tests on toxicological determinations, and taking into account the fact that we are dealing with two factors which are very different in sensitivity, the mother and the foetus. A substance which is not toxic to the mother can nevertheless result in malformations or death in the embryo.

Statistical comparison between the control and treated animals, the basis of all teratogenic studies, does not resolve all the problems, and its application is at times complicated.

In the present investigation we have considered parameters such as the increase in weight, the mothers producing affected foetuses, and the numbers or percentages of these in relation to implantations; this leads finally to the calculation of the teratogenic index.

Using these control criteria it is observed that Spirulina fed to three species of animals over three different periods during gestation, and at levels of 10, 20 and 30% in the diet, do not result in any teratogenic effects.

Checks carried out on the increase in weight, without any other special manipulation, indicate that the general state of the mothers fed on the alga is not modified, and that there is an absence of major abortion which could possibly have occurred during the course of gestation.

Therefore estimating the results on the one side for the mothers and on the other the risk for the foetuses in each litter, it can be seen that Spirulina does not cause changes during the gestation of the animals as would be indicated by malformations, anomalies or embryonic reabsorptions.

Some statistical differences in the mean weight of the foetuses, the number of implantations per fertile female or of foetuses per pregnant female, do not suggest any relationship with the alga since they constitute isolated cases which do not show any relationship to the level of Spirulina fed to the animals.

The absence of harmful effects from Spirulina in this study agrees with the other results obtained from studies of reproduction and lactation, carried out in this case with the rat.

GENERAL DISCUSSION AND COMMENTS

At the conclusion of this work the following analysis of the results can be arrived at.

Generally speaking it may be concluded that Spirulina, incorporated in the diet in concentrations of 10, 20 and 30%, does not produce changes in those parameters normally investigated in studies of sub-acute toxicity, toxicity over a long period, reproduction and lactation, mutagenesis and teratogenesis.

The significant differences observed in some cases do not follow any relationship with the doses given, and are therefore isolated cases. Since they were not reproduced in the final part of the experiment their effect cannot be attributed to Spirulina. The same applies to certain haematological and biochemical values on the serum in the tests on sub-acute and chronic toxicity, and in the multigeneration study of reproduction and lactation.

The data indicated by the studies of sub-chronic and chronic toxicity agree with those reported by other authors who employed different concentrations of the alga (Till and Willems, 1971) and those whose aims and objectives were different (Boudene et al. 1976). Other algae, such as Scenedesmus and Chlorella, however, have been the subject of short-term studies (Venkartaram et al., 1977, Yannai et al. 1979; Becker, 1978_a).

The multigeneration study of reproduction and lactation does not show any changes in the indices of fertility, gestation, viability and lactation over the three generations which were bred during a period of two years. In a study carried out on pigs by Février and Sève (1976), using Spirulina as a protein supplement at low dosages, reproduction was not affected, either from the point of view of size of litters or of the subsequent rate of growth of the animals. To the knowledge of the author, and with the exception of the work of Pabst (1978), no multigeneration studies have been carried out with algae, although this is considered important because of the information it can provide on possible effects on fertility in the male and female and effects during gestation, delivery and mutagenicity (FDA, 1970).

The tests on lethal dominants in the rat and mouse, which were carried out for the purpose of ascertaining the existence of mutagenic properties, show the activity of Spirulina as judged by the number of dead and live implantations.

The tests on teratogenicity, which were carried out on three animal species, showed that Spirulina fed at different concentrations and during four different periods of gestation does not cause any congenital malformations or embryonic reabsorptions.

The method of interpreting the data, separated between the mother and the foetuses, makes it possible to consider on

the one hand the risk to the mother of pursuing a normal gestation or otherwise, and on the other hand the risk for each foetus in the litter. This manner of interpretation is possible in multi-gestational species but not in monogestational species where the risks to the mother and the foetus are combined.

The results of the analysis for nucleic acids, metals, non-metals, pesticides and microbiological content indicate that Spirulina complies with the standards established in this respect by international organizations.

In regard to the nucleic acids it is known that a high level of these causes hyperuricemia with consequently gout, uricemic nephropathia and renal calculi. In addition to nutritional causes the ingestion of alcohol or drugs and sex and obesity are factors which influence its level (PAG, 1975).

The ingestion of 46 grams of Spirulina per day involves the consumption of 2 g of nucleic acids (Bourges et al., 1971) which, according to the PAG (1975), should be the limit since with another 2 g coming from all sources this would then not exceed 4 g of total daily ingestion by the adult. It should be pointed out, however, that unicellular proteins from algae are not ingested as the sole source of proteins but merely supplement and enrich basic diets (Becker, 1978_b). This fact reduces the possibility that the consumption of Spirulina could represent a risk in regard to nucleic acids.

In respect of the heavy metals and non-metals Spirulina contains, according to the latest determinations, concentrations of lead and arsenic which are lower than those recommended by the IUPAC (1974), being 5 ppm and 2 ppm respectively. Concentrations of mercury have varied between 0.01 and 0.2 ppm as against the standard of 0.1 ppm which they suggest.

The levels of cadmium on the other hand vary between 0.01 and 0.1. With an ingestion of 5000 g of Spirulina per week this would result in the consumption of the weekly limit of 0.5 mg which is recommended by the WHO/FAO (1972) for a 60 kg adult.

The reason for the difference between the determinations carried out by Boudene et al. (1976) and others who have subsequently repeated them could be due in part to the improvements in the technical conditions in the cultivation of the alga and also to the increased flow of water through the spiral channels as a result of the higher production. In fact periodical determinations of some metals have not shown any increase.

The presence in Spirulina of 3,4-benzopyrene, a general indicator of the presence of other carcinogenic materials in the group of aromatic policyclic hydrocarbons, is much lower than that in foodstuffs such as spinach, legumes, chicory, garlic, etc., (Truahut and Ferrando, 1976). Standard No. 15 of the PAG lays down the following margins for hydrocarbon residues: total residual hydrocarbons, 0.5%; total residual

aromatic hydrocarbons, 0.05%; benzopyrene, 5 µg/kg (PAG, 1974_b). Heptadecane for its part represents 65% of the total hydrocarbons which were found to be present. Taking into account the fact that the toxicity of these compounds is known, and considering the data which have been obtained from prolonged experiments, they do not appear to produce any phenomena of chronic toxicity (Tulliez et al., 1975).

In the case of other toxic materials, such as pesticides, the analyses carried out show only traces which do not indicate any risk to health, in accordance with existing knowledge; the World Health Organization (WHO, 1970) has fixed acceptable daily consumptions for some of these, and Spirulina meets these requirements.

The microbiological aspect of the cultivation and of the final product do not show any general contamination. The possible presence of antibacterial substances in Spirulina (Jacquet, 1976) could constitute a satisfactory guarantee for its innocuousness and for its storage over relatively prolonged periods. The process generally used for obtaining the alga is hygienically acceptable, whilst the installation of a pasteurisation unit will even further improve the sanitary conditions.

The combination of all the results obtained from these studies makes it possible to conclude that Spirulina does not produce

toxic or dangerous effects in laboratory animals, in those parameters studied and with the indicated levels.

In agreement with these results, and with the previous ones on the consumption of Spirulina by humans in remote times up to the present day, it is to be hoped that other clinical studies will demonstrate its innocuousness, and will probably produce similar data.

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