In this case ultrasonic diagnosis did not yield conclusive results at the first attempt.

The trophoblastic indices of hormonal activity did not behave pathologically.

On the contrary, α-foetoprotein which was abnormally and persistently high in the presence of a live embryo, was the only new factor that emerged from this investigation.

In the literature on the subject this is the first report of such a phenomenon; it markedly differs from the finding in threatened abortion and imminent death of the embryonic foetus.

These finding cannot be explained in detail on the basis of our present knowledge, and will require further confirmation.

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Effects of hydrocortisone and hydroxycobalamin on the rat and rabbit foetus

Experimental teratological study

by

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Our aim was to undertake an experimental study in order to verify whether a combination of drugs commonly used in therapeutic practice, such as a cortisone and a vitamin, might have an embryotoxic action when compared in two animal species such as the rat and the rabbit.

Our chief purpose was to discover whether the embryotoxic « threshold value » already recognized for the cortisones, could be affected by the presence of a vitamin (B12 or hydroxycobalamine in this instance), and whether the presence of the vitamin might positively or negatively alter the effect of the cortisone drug.

In view of the similarity of action of the many cortisones used in therapy, we chose disodium hydrocortisone-21-phosphate as representative of this group of drugs.

An experimental study of embryotoxicity was therefore undertaken, according to the method described by Cook, M. J. [1]. This experimental study was carried out on two animal species: the rat and the rabbit.

MATERIAL AND METHODS

RAT - Adult male and female albino rats of the Sprague-Dawley strain were used.

After pairing and when fertilization had occurred, a daily check was made

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by vaginal smear; the gravid females were subdivided into 6 experimental groups, which were treated with the substances listed in Table 1.

All the substances were administered in a volume of 2 ml of solution per kg body weight, at various concentrations.

The animals were kept in Tecniplast cages under standard stalling conditions, were fed on a commercial pelleted feed and had free access to water.

Treatment with the various substances was started the next day after fertilization (considered to be the first day of gestation) and was continued up to the 20th day, when the females were killed by dislocation of the cervical vertebrae, weighed, and the foetuses were obtained immediately.

The following parameters were taken into consideration when the gravid females were killed:

- increase in the mother's body weight during pregnancy
- number of live foetuses
- sex of the foetuses
- number of dead foetuses
- total weight and mean weight of the live foetuses
- number of reabsorptions.

Some of the live foetuses (about 3/5) were treated by the alizarin red method in order to show up the skeletal tissue, while the remaining 2/5 were decalcified in order to examine the soft tissues.

Malformations and anomalies both of the skeletal tissue and of the soft tissues were displayed and evalued using the methods described by Wilson ([2]), using a stereoscopic microscope.

As regards the skeleton, the presence, morphological appearance and consistency of all the centres of ossification of each individual foetus were examined, while examination of the soft tissues was carried out on successive sections of about

Table 1. *Treatment given to the rat.*

Group	No. of gravid rats	Treatment	Total dosage	Route of administration
1	10	Physiological solution 2 ml/kg	2 ml/kg	S.C.
2	10	Hydrocortisone (HC) 2.5 mg/kg + Hydroxycobalamina (OHB_{12}) 0.25 mg/kg	2.75 mg/kg	S.C.
3	10	Hydrocortisone (HC) 5 mg/kg + Hydroxycobalamina (OHB_{12}) 0.5 mg/kg	5.5 mg/kg	S.C.
4	10	Hydrocortisone (HC) 10 mg/kg + Hydroxycobalamina (OHB_{12}) 1 mg/kg	11 mg/kg	S.C.
5	10	Hydrocortisone (HC) 10 mg/kg	10 mg/kg	S.C.
6	10	Thalidomide 250 mg/kg		P.O.

1 - 1.5 mm, so as to display the normal morphology and integrity of the principal organs and systems such as the brain, eyes, heart, large vessels, lungs, liver, stomach, spleen, excretory apparatus and genital apparatus.

RESULTS

The results of the experiments are summarized in detail in Tables 2 - 8. From examination of these tables some conclusions can be draw on the data obtained, taking into account the type of pharmacological association used, after first briefly summarizing the data shown in the tables themselves.

Increase in the mother's body weight during gestation. As regards the finding relating to this parameter, a statistically significant difference was found to exist between the females treated with physiological solution or thalidomide and those treated with a combination of cortisone and vitamin (HCOH/B12) and with hydrocortisone (HC). In the females treated with the latter substances, and particularly in those treated with higher doses (5.5 and 11 mg/kg), the increase in body weight was substantially less, while there was no difference between the females treated with HCOH/B12, 2.75 mg/kg, and the control females.

Without any exceptions the treated females proceeded regularly to term and, except for the diminished increase in body weight, they showed no pathological signs.

Number of living foetuses. There was no change in the number of living foetuses as a result of the treatment undergone by the gravid females during gestation.

Number of dead foetuses. From examination of the findings it can be deduced that during the course of this trial only the groups treated with HC, 10 mg/kg, and with thalidomide, 250 mg/kg, showed any definite foetal mortality.

Even though this mortality was no different as regards statistical significance from that usually encountered in untreated animals, it was found in this case that no fatalities were recorded either among the control animals or in those treated with the HCOH/B12 combination.

It would appear that the hydroxycobalamine component helps to mantain the vitality of the foetuses.

Sex of the foetuses. From all the findings reported it seems that the treatment given to the gravid females had no effect on this parameter.

Weight of the foetuses. Neither the total nor the mean weight of the live foetuses in the control group, in those treated with HCOH/B12, 2.75 and 5.5 mg/kg, or those treated with thalidomide, differed in any statistically significant manner from the weights of foetuses from mothers treated with HCOH/B12, 11 mg/kg, or with HC, 10 mg/kg. It was found that the mean weight of the latter foetuses was the lowest of all those in the different experimental groups.

Number of reabsorptions. Although it was not possible to make a statistical assessment of the data, because of their small number, it was evident that the mean number of reabsorption was greater in the animals of the groups treated with thalidomide or with HC.

The values obtained in the animals treated with the HCOH/B12 combination were all comparable with one another and with those obtained with the control animals, thus confirming the favourable effect of the association with HC alone.

Anomalies and malformations of the skeleton and tissues. One of the commonest findings on examination of the skeletons of laboratory animals treated

Table 2. *Teratogenesis tests on rats control group.*

No.	% increase in body weight	No. of live foetuses			Total weight of live foetuses g.	Mean weight of live foetuses g.	No. of dead foetuses g.	No. of reabsorptions	Foetuses with anomalies	
		M	F	T					Skeletal *	Tissular **
1	36.8	8	7	15	72.56	4.84	—	—	1(9)	1(6)
2	35.9	6	8	14	75.72	5.41	—	—	2(9)	0(5)
3	27.3	4	4	8	33.88	4.24	—	—	3(6)	1(2)
4	32.4	7	7	14	71.78	5.13	—	—	1(9)	0(5)
5	40.1	6	5	11	55.45	5.04	—	—	0(7)	1(2)
6	37.0	5	3	8	45.25	5.66	—	—	0(5)	0(5)
7	38.2	7	9	16	72.72	4.90	—	—	1(10)	0(4)
8	39.7	7	7	14	66.55	4.75	—	—	3(9)	0(3)
9	29.1	6	5	11	51.34	4.67	—	—	0(7)	0(4)
10	35.6	6	7	13	48.23	3.71	—	—	0(8)	0(5)
$\bar{X}$	35.25	6.25	6.25	12.5	59.35	4.84	0.0	0.25	14.0*	12.0*
D.S.	±4.31	±0.45	±0.72	±1.10	±14.45	±0.56				

* Mean of percentages. ** The numbers between brackets indicate the number of foetuses examined.

Table 3. *Teratogenesis tests on rats treated with HC+HCOH/B12. Group treated with HC 2.5 mg/kg+HCOH/B12 0.25 mg/kg s.c.*

No.	% increase in body weight	No. of live foetuses			Total weight of live foetuses g.	Mean weight of live foetuses g.	No. of dead foetuses g.	No. of reabsorptions	Foetuses with anomalies	
		M	F	T					Skeletal	Tissular
11	11.73	3	4	7	34.23	4.89	0	—	0(5)	0(2)
12	12.75	3	3	6	31.79	5.30	0	—	0(4)	0(2)
13	24.48	6	8	14	67.57	4.83	0	—	1(8)	0(6)
14	27.85	8	5	13	59.85	4.60	0	2	4(7)	0(6)
15	19.23	6	4	10	43.89	4.39	0	—	0(7)	0(3)
16	20.32	7	7	14	60.78	4.34	0	—	2(8)	0(6)
17	16.86	4	3	7	35.37	5.05	0	2	2(5)	0(2)
18	38.91	8	10	18	85.03	4.72	0	1	3(11)	0(7)
19	29.34	6	7	13	59.88	4.61	0	—	1(9)	0(4)
20	36.41	8	6	14	68.34	4.88	0	—	2(9)	0(5)
$\bar{X}$	23.79	±5.88	±5.87	±11.6	54.67	4.76	0.0	0.5	20.0*	0.0*
D.S.	9.33	±0.69	±0.89	±1.24	±17.63	±0,29				

* Mean of percentages. ** The numbers between brackets indicate the number of foetuses examined.

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Table 4. *Parameters noted at end of gestation in rats treated with HAC 5 mg/kg+HCOH/B12 0.5 mg/kg s.c.*

No.	% increase in body weight	No. of live foetuses			Total weight of live foetuses g.	Mean weight of live foetuses g.	No. of dead foetuses g.	No. of reabsorptions	Foetuses with anomalies	
		M	F	T					Skeletal	Tissular
21	22.4	6	7	13	54.61	4.20	0	0	0(7)	0(6)
22	12.5	4	5	9	34.27	3.81	0	0	0(5)	0(4)
23	12.4	4	5	9	41.22	4.58	0	0	0(6)	0(3)
24	22.5	6	6	12	42.44	3.54	0	0	0(7)	0(5)
25	17.2	6	5	11	44.17	4.02	0	2	3(7)	0(4)
26	16.2	5	6	11	43.53	3.96	0	0	2(7)	0(4)
27	18.2	7	7	14	56.01	4.00	0	1	1(8)	0(6)
28	14.9	6	7	13	51.21	3.94	0	2	0(6)	1(5)
29	11.7	6	4	10	43.40	4.34	0	0	2(6)	0(4)
30	19.4	4	10	14	48.83	3.49	0	0	4(8)	0(7)
X	16.74	5.40	6.20	11.60	45.97	3.99	0.0	0.33	17.0*	2.0*
D.S.	±3.95	±1.07	±1.69	±1.90	±6.66	±0.33				

* Mean of percentages. ** The numbers between brackets indicate the number of foetuses examined.

Table 5. *Parameters noted at end of gestation in rats treated with HC 10 mg+HCOH/B12 1 mg/kg s.c.*

No.	% increase in body weight	No. of live foetuses			Total weight of live foetuses g.	Mean weight of live foetuses g.	No. of dead foetuses g.	No. of reabsorptions	Foetuses with anomalies	
		M	F	T					Skeletal	Tissular
31	30.29	6	7	13	45.08	3.47	0	0	3(8)	
32	18.8	5	6	11	33.40	3.04	0	0	5(8)	
33	30.3	4	4	8	51.26	6.41	0	0	0(5)	
34	24.2	4	5	9	39.40	4.38	0	0	5(6)	
35	27.3	6	6	12	51.71	4.39	0	0	5(8)	
36	26.0	8	6	14	46.34	3.31	0	0	7(9)	
37	22.9	6	7	13	48.29	3.71	0	0	8(9)	
38	17.4	6	5	11	41.17	3.74	0	0	6(7)	
39	14.0	7	7	14	39.02	2.79	0	0	9(9)	
40	13.1	5	5	10	37.12	3.71	0	0	7(7)	
$\bar{X}$	22.42	5.7	5.8	11.5	43.38	3.50	0.0	0.0	70.0*	2.64*
D.S.	6.31	±1.25	±1.03	±2.07	±6.35	±1.79			31.0*	

* Mean of percentages. ** The numbers between brackets indicate the number of foetuses examined.

Table 6. *Parameters noted at end of gestation in rats treated with HC 10 mg/kg s.c.*

No.	% increase in body weight	No. of live foetuses			Total weight of live foetuses g.	Mean weight of live foetuses g.	No. of dead foetuses g.	No. of reabsorptions	Foetuses with anomalies	
		M	F	T					Skeletal	Tissular
41	18.9	5	6	11	35.5	3.23	0	0	7(7)	0(4)
42	11.1	4	5	9	27.1	3.01	0	0	5(5)	1(4)
43	14.3	7	7	14	38.4	2.74	0	0	9(9)	0(5)
44	13.2	5	5	10	46.2	4.62	1	0	7(7)	0(3)
45	16.8	5	6	11	31.7	2.86	0	3	7(8)	0(3)
46	19.0	5	7	13	34.9	2.91	2	1	8(8)	1(4)
47	16.4	4	5	9	37.5	4.17	1	2	4(6)	0(3)
48	22.6	6	7	13	39.4	3.03	0	0	5(9)	0(4)
49	11.7	7	4	11	33.2	3.02	0	1	6(7)	0(3)
50	20.1	6	6	12	27.1	2.26	0	0	8(8)	1(4)
$\bar{X}$	16.41	5.40	5.60	5.60	35.1	3.19	0.4	0.7	88.0*	8.0*
D.S.	±3.80	±1.07	±1.05	±1.05	±5.79	±0.69				

* Mean of percentages. ** The numbers between brackets indicate the number of foetuses examined.

Table 7. *Teratogenesis tests on rats treated with thalidomide 250 mg/kg orally.*

No.	% increase in body weight	No. of live foetuses			Total weight of live foetuses g.	Mean weight of live foetuses g.	No. of dead foetuses g.	No. of reabsorptions	Foetuses with anomalies	
		M	F	T					Skeletal	Tissular
51	42.6	6	7	13	60.45	4.65	0	0	4(7)	5(6)
52	11.7	5	4	9	29.52	3.28	0	2	5(5)	3(4)
53	54.2	5	5	10	43.10	4.31	0	0	3(6)	4(4)
54	65.8	7	7	14	57.68	4.12	0	0	6(8)	5(6)
55	66.5	7	6	13	64.48	4.96	0	2	7(8)	5(5)
56	58.1	6	4	10	50.10	5.01	0	2	4(6)	4(4)
57	64.2	5	4	9	40.23	4.47	3	1	3(5)	4(4)
58	30.6	4	5	9	42.48	4.72	4	0	2(5)	3(4)
59	56.3	6	6	12	57.60	4.80	0	0	3(7)	3(5)
60	59.9	8	6	14	57.82	4.13	0	0	5(8)	4(6)
$\bar{X}$	50.99	5.90	5.40	11.30	50.35	4.45	0.70	0.70	64.2	84.3
D.S.	±17.74	±1.20	±1.12	±2.11	±11.13	±0.52			19.0	15.1

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Table 8. *Control of teratogenesis in rats treated with HCOH/B_{12}. Summary table of values found at end of gestation.*

	No. of gravids rats treated	% increase of body weight	No. of live foetuses			Mean weight of litter g.	Mean wight of live foetuses g.	No. of dead foetuses	No. of reabsorptions	Percentage of foetuses with anomalies	
			M	F	T					Skeletal	Tissular
Physiol. soln 2 ml/kg	10	35.25 ±4.31	6.25 ±0.45	6.25 ±0.72	12.5 ±1.10	59.35 ±14.45	4.84 ±0.56	0.0	0.25	14.0 *	12.0 *
HCOH/B_{12} 2.25 mg/kg S.C.	10	23.79 ±9.33	5.88 ±0.69	5.87 ±0.89	11.6 ±1.24	54.67 ±17.63	4.76 ±0.29	0.0	0.5	20.0 *	0.0 *
HCOH/B_{12} 5.50 mg/kg S.C.	10	16.74 ±3.95	5.40 ±1.07	6.20 ±1.69	11.6 ±1.90	45.97 ±6.66	3.99 ±0.33	0.0	0.33	17.0 *	2.0 *
HCOH/B_{12} 11 mg/kg S.C.	10	22.42 ±6.31	5.7 ±1.25	5.8 ±1.03	11.5 ±2.07	43.38 ±6.35	3.50 ±0.79	0.0	0.0	70.0 *	2.64*
HCOH/B_{12} 10 mg/kg S.C.	10	16.61 ±3.80	5.40 ±1.07	5.60 ±1.05	11.0 ±1.52	35.1 ±5.79	3.19 ±0.69	0.4	0.7	88.0 *	8.0 *
Thalidomide 250 mg/kg S.C.	10	50.99 ±17.74	5.90 ±1.20	5.40 ±1.20	11.30 ±2.11	50.35 ±11.13	4.45 ±0.52	0.7	0.7	64.2 *	84.3 *
Analysis of variance	g. 1 F. P.	5/55 15.32 <0.001				5/55 5.81 <0.01	5/55 27.37 <0.001			5/55 9.45 <0.001	

* Mean of percentages.

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with drugs is their retarded maturation, characterized by a lesser degree of calcification.

Among the foetuses from the groups treated with HCOH/B12, 11 mg/kg, with HC, 10 mg/kg, and with thalidomide, 250 mg/kg, these parameters were very often altered, and in particular with regard to the extremities, the sternum, the cranial bones (specially the parietal).

True malformations were also found in these foetuses, that is, changes sufficient to hinder the animal's normal development, while in the control animals and in those treated with HCOH/B12, 2.75 and 5.50 mg/kg, the animals did not go beyond the level of retarded skeletal maturation.

Examining the findings in detail it can be stated that the major quantitative injuries were produced by hydrocortisone at a dose of 10 mg/kg; in the foetuses of this group, in fact, the combined effects of both the embryotoxic action of the cortisone given alone, and of the slight calcification consequent on its pharmacological action, were very much in evidence. The majority of the foetuses involved had a type of lesion that was markedly uniform.

Very similar, though less in number, were the abnormalities found in the foetuses of gravid females treated with HCOH/B12, 11 mg/kg.

In this group the injuries due to the reduced calcification were slightly less, and the flat bones, though they showed some immature characteristics, were more calcified than the corresponding bones belonging to females treated only with hydrocortisone.

As regards the foetuses from mothers treated with thalidomide, 250 mg/kg orally, they had more uniform lesions, characteristic of the type of drug. These lesions more or less obviously affected all the foetuses, but occurred with extreme seriousness in one litter.

In this series of foetuses, some of the malformations noticed were curvature of the tail, absence of one or more cranial bones, especially the occipital, and secondarily the parietal, absence of fusion of the arches of the vertebrae, and asymmetry in the sequence of the ribs.

As regards the soft tissues, some of the anomalies observed were: omphalocele, abnormal dilatation of the cerebral ventricles, one case of agenesia of the interventricular septum, ectopia of the stomach and kidneys, and a few cases of occlusion of the ureters.

CONCLUSIONS

The trials were carried out on one animal species (rat) which is definitely sensitive to a teratogenic agent such as thalidomide.

Hydrocortisone, when given alone at a dosage of 10 mg/kg s.c. throughout gestation, brough about some changes in the foetuses, as was to be expected in view of the particular type of pharmacological action that it exerts, and these changes, different from those induced by thalidomide, were the most marked of all the groups.

A certain type of toxic action, which also affected the mothers, must not be forgotten here; this became evident on examining the increases in body weight which, in the groups that received HC, was less than in the control animals and in those treated with thalidomide.

It was evident from examination of the foetuses that the lesions encountered in the foetuses of females treated with HCOH/B12, 11 mg/kg, were less serious

and more constant than those met with in the foetuses treated with HC alone.

Descending the scale of dosages administered to the gravid females, it can be noted that the injurious effects on the foetuses gradually decreased in intensity, severity and frequency, while a dosage between 2.5 and 5.50 mg/kg of HCOH/B12 (in the proportions used by us) can certainly be defined as a « threshold value » below which the anomalies are superimposable upon and indistinguishable form those found in the control animals.

MATERIAL AND METHODS

RABBIT - The trials with the substances HC and HCOH/B12 were also repeated on the rabbit, using the same procedure.

The reason for choosing this second animal species was chiefly its particular sensitivity to teratogenic agents, even though this is usually shown as a high percentage of spontaneous malformations ([3]).

For this research, 30 white New Zealand rabbits were used. After fertilization, they were subdivided into groups of 5 animals each treated from the 2nd to the 29th day of gestation with the various forms of treatment listed in Table 9. After normal pairing with males of the same strain, the females were placed in individual hutches under standard stalling conditions, with free access to food (commercial pelleted feed) and water. They were left alone except for cleaning and the treatment which began the day after mating and continued until the 29th day for gestation. On the 29th day the females were killed by trauma to the back of the neck (to avoid any toxic effect of anaesthetics upon the foetuses)

Table 9. *Treatment given to the rabbit.*

Group	No. of animals	Treatment	Total dosage	Route of administration
1	5	Physiological solution 0.5 ml/kg	0.5 ml/kg	S.C.
2	5	Hydrocortisone (HC) 2.5 mg/kg + Hydroxycobalamina (OHB_{12}) 0.25 mg/kg	2.75 mg/kg	S.C.
3	5	Hydrocortisone (HC) 5 mg/kg + Hydroxycobalamina (OHB_{12}) 0.5 mg/kg	5.5 mg/kg	S.C.
4	5	Hydrocortisone (HC) 10 mg/kg + Hydroxycobalamina (OHB_{12}) 1 mg/kg	11 mg/kg	S.C.
5	5	Hydrocortisone (HC) 10 mg/kg	10 mg/kg	S.C.
6	5	Thalidomide 150 mg/kg	150 mg/kg	S.C.

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and, after checking the body weight, the foetuses were obtained, taking the following parameters into consideration: increase in body weight of the gravid females; total number of implantations; number of live foetuses; number of dead foetuses; number of reabsorptions; total weight of live foetuses; mean weight of live foetuses.

About 2/3 of the foetuses, after removal of the skin and evisceration, were macerated and treated so as to transilluminate the soft tissues and to display the skeleton by the alizarin red method (a stain derived from anthraquinone, with an elective affinity for bone tissue).

The remaining foetuses were decalcified so as to prepare the soft tissues for careful examination, after having made successive sections of about 2 mm thickness for each foetus. The soft tissues were examined by Wilson's technique ([2]), which consists in demostrating, by means of lenses and a stereoscopic microscope, the integrity or otherwise of the principal organs such as the olfactory bulbs, the eyeballs, cerebral ventricles, the various structures of the brain, the interventricular septum of the heart, the principal vessels, lungs, stomach, liver, kidneys and ureters, and genital organs.

RESULTS

The results obtained from the trial are summarized in detail in Table 10 - 16. Before considering these it should be mentioned that, perhaps because of the powerful immunosuppressor effect of cortisone, the animals (specially the treated animals) were affected by various infections which were cured by therapeutic doses of antibiotics. It cannot therefore be stated that the trial was carried out under the best conditions.

Nevertheless, it seems worth while to proceed to examine the data obtained.

Increase in body weight of the gravid females. The marked sensitivity of the rabbit to cortisone treatment is shown in relation to this parameter, in strict correlation with the dosage of the substances received; the diminution of the values was practically dose-dependent. It was found that the dose of HCOH/B12, 2.75 mg/kg, did not produce a very consistent reduction in the increased body weight of the mothers; values close to those of the controls were recorded. Thalidomide was found to be totally inactive in this respect. This, as we know, has no toxic effects on the adult body but only has a teratogenic action on the foetus.

Number of implantations. As regards this parameter, it does not seem possible to establish any correlations with the dosage; probably this resulted from the unsuitable experimental conditions. At any rate it was noted, both in the controls and in the group treated with thalidomide (150 mg/kg), that higher values were found, while the variability was marked in the different groups treated with HC or HCOH/B12. In this case too, it should be mentioned that the group treated with HCOH/B12, 2.75 mg/kg, showed similar values, though they were slightly less than those of the control group.

Number of live foetuses. This parameter seems to have affected in a way determined by the treatment, in that the number of live foetuses diminished as the dose increased, in the groups treated with HC and HCOH/B12, but it showed no variations (the results were, in fact, superimposable) in the control group and the group treated with thalidomide, 150 mg/kg.

Number of dead foetuses and of reabsorptions. While it is not apparently possible to distinguish the real variations of these two parameters among the various

Table 10. *Teratogenesis tests on the rabbit treated with HC and HCOH/B12. Control group treated with physiological soln. 2 ml/kg s.c.*

No.	% increase in body weight	No. of implanta-tions	No. of live foetuses	No. of dead foetuses	No. of reabsorp-tions	Total weight live foetuses	Mean weight dead foetuses	Foetuses with variations	
								Skeletal	Visceral
1	9.20	10	10	1	0	467	46.7	3(6)	0(4)
2	8.30	7	7	0	0	346	49.4	1(4)	0(3)
3	12.4	9	8	0	0	497	62.1	1(5)	0(3)
4	6.8	12	11	0	0	481	43.7	2(7)	0(3)
5	22.1	8	8	0	2	425	53.1	0(5)	0(4)
$\bar{X}$	11.76	9.20	8.80	0.20	0.40	443.2	51.0	25.0	0.0
±E.S.	2.74	0.86	0.73			27.1	3.2	8.0	

Table 11. *Teratogenesis tests on the rabbit treated with HC. 2.5 mg/kg+HCOH/B12 0.25 mg/kg s.c.*

No.	% increase in body weight	No. of implanta-tions	No. of live foetuses	No. of dead foetuses	No. of reabsorp-tions	Total weight live foetuses	Mean weight dead foetuses	Foetuses with variations	
								Skeletal	Visceral
6	3.70	11	9	2	0	310	34.5	0(5)	1(4)
7	12.24	6	4	0	2	103	25.8	1(2)	0(2)
8	5.10	8	6	0	2	187	31.2	2(4)	0(2)
9	11.45	8	5	1	2	139	27.3	1(3)	1(2)
10	9.87	9	7	2	0	253	36.1	1(3)	0(2)
$\bar{X}$	8.47	8.40	6.20	1.0	1.20	198.4	31.1	%30	%15
±E.S.	1.70	0.81	0.86			37.55	1.93	5	

Table 12. *Teratogenesis tests on the rabbit treated with HC 5 mg/kg+HCOH/B12 0.5 mg/kg s.c.*

No.	% increase in body weight	No. of implanta-tions	No. of live foetuses	No. of dead foetuses	No. of reabsorp-tions	Total weight live foetuses	Mean weight dead foetuses	Foetuses with variations	
								Skeletal	Visceral
11	8.45	3	3	0	0	84.6	28.2	2(2)	0(1)
12	5.40	N.G.	—	—	—	—	—	—	—
13	9.35	N.G.	—	—	—	—	—	—	—
14	3.70	8	6	0	2	115.8	19.3	3(4)	1(2)
15	7.80	9	6	2	1	135.6	22.6	3(4)	2(2)
$\bar{X}$	6.94	6.67	5.0	0.67	1.0	112.0	23.4	%83	%50
±E.S.	1.04	1.44							

N.G. = Non gravid.

Table 13. *Teratogenesis tests on the rabbit treated with HC 10 mg/kg+HCOH/B12 1 mg/kg s.c.*

No.	% increase in body weight	No. of implanta-tions	No. of live foetuses	No. of dead foetuses	No. of reabsorp-tions	Total weight live foetuses	Mean weight dead foetuses	Foetuses with variations	
								Skeletal	Visceral
16	3.90	N.G.	—	—	—	—	—	—	—
17	4.50	3	0	0	3	—	—	—	—
18	6.20	6	2	0	4	49.6	29.8	2(2)	—
19	2.15	4	0	0	4	—	—	—	—
20	3.72	5	0	0	5	—	—	—	—
$\bar{X}$	2.29	4.50	0.50	0.0	4.0	49.6	29.8	%100	
±E.S.									

N.G. = Non gravid.

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Table 14. *Teratogenesis control on the rabbit treated with HC at dose of 10 mg/kg s.c.*

No.	% increase in body weight	No. of implantations	No. of live foetuses	No. of dead foetuses	No. of reabsorptions	Total weight live foetuses	Mean weight dead foetuses	Foetuses with variations	
								Skeletal	Visceral
21	4.22	6	0	2	4	—	—	—	—
22	5.10	4	0	4	0	—	—	—	—
23	3.18	5	0	0	5	—	—	—	—
24	2.75	7	2	2	3	37.4	18.7	2(2)	—
25	4.60	9	5	4	0	89.5	17.9	2(3)	2(2)
X	0.24	6.20	1.40	2.40	2.40	63.45	18.30		%100
±E.S.									

Table 15. *Teratogenesis control on the rabbit treated with thalidomide 150 mg/kg orally.*

No.	% increase in body weight	No. of implantations	No. of live foetuses	No. of dead foetuses	No. of reabsorptions	Total weight live foetuses	Mean weight dead foetuses	Foetuses with variations	
								Skeletal	Visceral
26	22.4	10	10	0	0	513	51.3	6(6)	4(4)
27	18.3	8	7	0	0	348	49.7	4(4)	3(4)
28	19.6	13	9	4	0	383	42.6	4(6)	3(3)
29	12.8	9	7	0	2	339	48.4	3(4)	2(3)
30	17.5	11	10	1	0	398	39.8	6(7)	2(3)
X	18.12	10.2	8.6	1.0	0.4	396	46.4	%85.0	86.7
±E.S.	1.57	0.86	0.68			31.1	2.2		8.1

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Table 16. *Summary table of values found in the rabbit.*

Treatment	No. of rabbits treated	% increase of body weight	No. of implantations	No. of live foetuses	No. of dead foetuses	No. of reabsorption	No. of gravid rabbits examined	Total weight of foetuses	Mean weight of live foetuses	No. of foetuses with variations	
										Skeletal	Tissular
Physiol. soln. 0.5 ml/kg S.C.	5	11.76	9.20	8.80	0.20	0.40	5	443	51.0	25.0	0.0
HC 2.5 mg/kg OHB_{12} 0.25 mg/kg	5	8.47	8.40	6.20	1.0	1.20	5	198	31.1	30	15
HC 5 mg/kg OHB_{12} 0.5 mg/kg	5	6.94	6.67	5.0	0.67	1.0	3	112	23.4	83	50
HC 10 mg/kg OHB_{12} 1 mg/kg	5	2.29	4.50	0.50	0.0	4.0	4	49.6	29.8	100	—
HC 10 mg/kg	5	0.24	6.20	1.40	2.40	2.40	5	63.4	18.30	100	100
Thalidomide 150 mg/kg P.O.	5	18.12	10.2	8.6	1.0	0.4	5	396	46.4	85	86

groups, the values obtained in the groups treated with HC, 10 mg/kg, and with HCOH/B12, 11 mg/kg, will be considered separately. In these two groups the action of the cortisone was so powerful that the presence or otherwise of the vitamin was not felt to the slightest extent. In both these groups, in fact, there were few, if any, live foetuses and a number of dead foetuses and reabsorptions which corresponded to almost the total number of implantations. This action, on a species so sensitive to cortisone, seems certainly attributable to the immunosuppressor effect.

Total weight and mean weight of the live foetuses. These parameters were influenced by the treatment; the weight of the foetuses, which was similar in those of the control group and the group treated with thalidomide, was however diminished as the dosage of HCOH/B12 was increased. The lowest weight was found in the group treated with hydrocortisone alone, 10 mg/kg.

Examination of the abnormalities. The abnormalities and variations, both skeletal and tissular, that we found, were clearly different as between the group treated with thalidomide and the others. In the first group we found the enormous range of malformations caused by this drug, including numerous cases of acephalia, exencephalia, ectopia of various organs and parenchyma, torsion of the limbs, phocomelia, agenesia of the ureters and various others of lesser importance (Fig. 1, 2, 3, 4), while in the groups treated with cortisone, anomalies were noted especially in the skeleton; these were more serious in the groups treated with

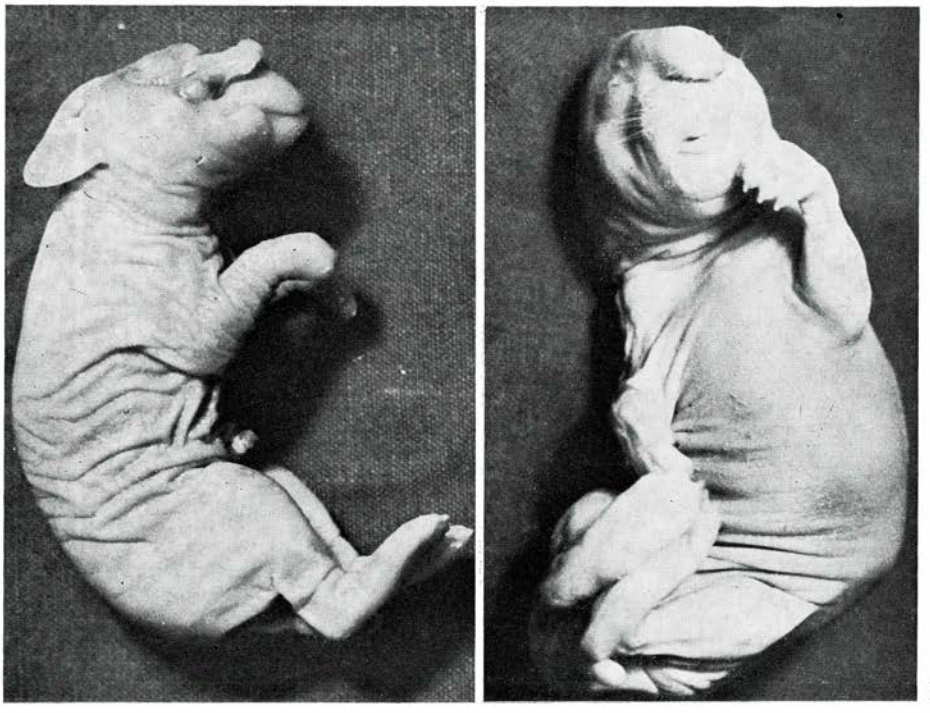

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FIG. 1 - Foetus of rabbit treated with thalidomide 150 mg/kg, orally. Agenesia of telencephalon; partial torsion of limbs and omphalocele.

FIG. 2 - Foetus of rabit treated with thalidomide 150 mg/kg orally. Partial agenesia of encephalon and eyeballs. Torsion of fore limbs.

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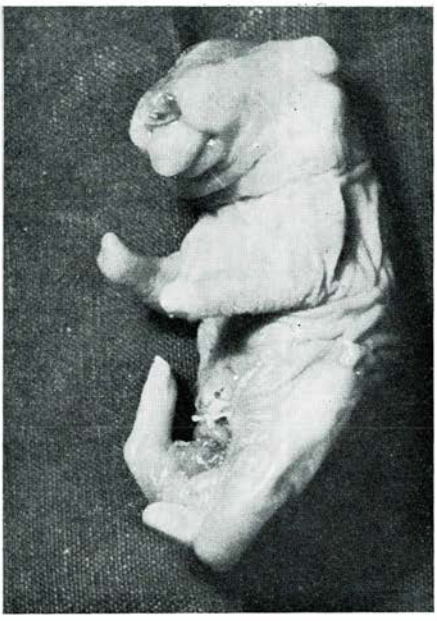

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FIG. 3 - Foetus of rabbit treated with thalidomide 150 mg/kg orally. Both skeletal and visceral multiple malformations. Those visible are: acephalia, phocomelia, omphalocele, with exteriorization of part of small intestine.

FIG. 4 - Foetus of rabbit treated with thalidomide 150 mg/kg orally. Multiple malformations, the following being visible: acephalia, macroglossia, partial phocomelia, omphalocele, etc.

higher doses of the substances being treated, and less so in the others, though the same percentage incidence was found in the latter.

CONCLUSIONS

It should be remembered that the morbid condition in which the animals found themeselves during gestation may also have produced some toxic action upon them. Thus it is difficult to make correlations between the incidence and severity of the anomalies, malformations and dosages.

The rabbit is usually a carrier of various parasitic deseases, which often results in illness of a parasitic or infective type such as coccidiosis and pasteurellosis. When the animal is in a state of immunosuppression, as is the case during cortisone treatment, these infective forms are diffused in epizootic form, and also affect the control animals. In order to bring such a burdensome trial to a conclusion, it was necessary to treat the mothers with antibiotics and sulphonamides, and this, given the sensitivity of the species, may have contributed to the interference with the regular development of the foetuses.

However, even if with the reservations already expressed, it seems possible to conclude that this experiment on the rabbit has once more confirmed the teratogenic action of thalidomide; that hydrocortisone produces its embryotoxic effects predominantly in the skeleton and very probably in correlation with the administered dosage; that in the case of the rabbit too, there is a « threshold value » for the substances examined, which may be established at 2.75 mg/kg of HCOH/

B12 (in the proportions used by us), below which the anomalies or variations encountered are completely superimposable upon those of the control animals in the species used.

DISCUSSION

Even though the trial on the rabbit was not carried out under optimal conditions, due to the combined pharmacological and toxic effect of hydrocortisone on the maternal body, some differences were still noted between the action of hydrocortisone alone and its action when combined with vitamin B12. In fact the action produced by HCOH/B12 on the embryo resulted (especially in the rat and at doses much higher than those given therapeutically) in a lower incidence of embryotoxic effects, but when doses of cortisone at the limiting level for normal development of the embryo were given, the injuries caused by hydrocortisone were on average more numerous and serious than those caused by HCOH/B12. It seems worth repeating that results obtained in the absence of strict experimental control are very hazardous. During the present trial, for example, the basic values for the anomalies obtained in the rabbit were much higher than they usually are for this species, showing not only a marked variability between one species and another ([4]) but even within the same species, with different drugs.

The results obtained confirmed the embryotoxic action of the cortisones ([5-6]), while the fact that they were demonstrated with the cortisone examined for « threshold values » in comparing embryotoxicity, may explain the difference existing between those who have found phenomena of this kind in the pregnant woman ([7, 8, 9, 10, 11, 12]) and those who have not observed any signs of embryonal toxicity after the administration to pregnant women of cortisones at therapeutic doses ([7, 10, 13, 14, 15, 16]).

It seems, from the trial as a whole, that the most striking finding is that of the existence of a « threshold value » for the embryotoxic effect of hydrocortisone, above which the effects encountered are in strict correlation with the dose administered. It does not appear that the addition of vitamin, which is often associated with cortisones, has any real importance with regard to the effects on the foetus directly, but it does seem to exert a satisfactory action upon the maternal body, which may consequently better explain the trophic effects on the foetus. The parameter positively affected by the presence of the vitamin was in fact the body weight of the foetuses, but not the parameter of possible anomalies or malformations. This is due to the fact that hydroxycobalamine antagonizes the proteo-catabolic action of the cortisones.

The results of this research also confirm that hydrocortisone (and certainly also its more powerful synthetic derivatives) ([15]) has no teratogenic action when used in therapeutic doses.

In the present trial, in fact, the dosage corresponding to the « threshold value » for embryotoxicity was about 7 times greater than that intended for therapeutic use (2.50 mg/kg against 0.35 mg/kg).

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Alpha-foetoprotein in threatened abortion

by

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The prognosis of threatened abortion is still an unsolved problem, nor is it often easy to determine its cause, at least before the abortion occurs. The methods hitherto used have been confined to determining the hormonal production of the trophoblast (HCG, HPL, E_3) by direct or indirect methods such as the study of the vaginal receptor. One new prospect could be that of a non-trophoblastic index, such as alpha-foetoprotein, which is produced by the embryonic foetal liver and by the yolk sac.

The purpose of this study is to evaluate the validity of the determination of alpha-foetoprotein as a prognostic index in threatened abortion and to make a comparison with the data already present in the literature.

MATERIAL AND METHODS

Plasma alpha-foetoprotein was determined in 108 pregnant women between the 7^{th} and 21^{th} week, clinical signs of threatened abortion being present.

At the same time, a control group of 123 pregnant women were examined in which gestation proceeded entirely normally to term.

The plasma determinations were done by the radio-immunological method (double antibody) using the kit made available by the Sorin company.

RESULTS

Concentration of alpha-foetoprotein in normal pregnancies

Because of the skewed distribution of results, was not calculated the mean and standard deviation but was used a non-parametric method based on centiles, the arithmetic mean being unduty increased by few wildly high results. The centiles were approximated in the least square by a polynomial of 3^{rd} degree.

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