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Effect of Cortisone on the Foetus of Pregnant Rats

An attempt at widening the concept of teratogenesis

By

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Since the consequences of thalidomide treatment had become known, the possible teratogenic effects of drugs have been studied with especial care. New preparations are now carefully tested in animal experiments from a teratological angle. A number of methods [14] makes it possible to induce congenital malformations in certain animals by certain doses of certain drugs. Standards on the basis of which any given drug should be classified as teratogenic for human subjects have likewise been elaborated, but they are still unsatisfactory. Numerous problems have to be solved in this respect, for there is no animal whose placenta would select drugs in the same way as the human placenta does and also because the assimilation of the same drug may be different in humans and in animals [15] so that its effect on the foetus may vary accordingly.

In the knowledge of the current teratologic tests we feel that even the very definition of teratogenesis, i. e. the range covered by this term, requires further elucidation. The usual methods reveal only gross or microscopical congenital anomalies while biochemical changes and irregularities which manifest themselves later but are imperceptible at birth (e. g. enzymopathies) escape notice. There are institutions, e.g. the USA Food and Drug Administration, which require [3] that, after having been treated before or during pregnancy, the animals be kept under observation for a long time after having thrown their young and that the rate of growth of the offspring be registered: this kind of testing method is, however, not universally adopted. The present experiments were, therefore, designed to observe the postnatal life of animals whose mothers had been treated with cortisone during pregnancy and thus to contribute to a more precise definition of drug-induced teratogenesis.

This study forms part of experiments initiated by the Hungarian Pharmaceutical Research Institute for the elaboration of new teratological tests.

Material and Method

Wistar rats of our own stock were used. Adult, sexually mature females of about 200 g body weight were mated and the occurrence of pregnancy verified by controlling the vaginal smear every day. Pregnant animals were then separated and 13 of them given intramuscular injections of 15 mg cortisone acetate daily on the 12th 13th, 14th and 15th days of pregnancy. Treatment was tolerated well and littering occurred on the 21st day, similarly as with the controls. To facilitate comparison, the test animals and the controls were selected in pairs so that — as far as possible — each test and its control littered on the same day. This in fact occurred in all but three cases; three controls had no counterparts among the tests in two cases, and one control had two test counterparts in the third case.

The number of littermates and the individual weights of the newborn animals were registered and then all young animals weighed every third day. Disregarding deaths in the first three extrauterine days, the deaths were registered up to the 45th postnatal day when the survivors were killed, their thymus and spleen weighed, and both organs together with a lymph node fixed and embedded in paraffin for histological study. Sections were stained with haematoxylin-eosin, toluidine blue, methylgreen-pyronine and Giemsa's dye. Differences between test and control animals in the number of littermates, birth weight, growth and mortality rates, weight of thymus and spleen on the 45th day, were analysed mathematically.

Results

The organs removed on the 45th day of extrauterine life presented no uniform picture. In the test animals the medulla of the thymus was slightly enlarged, their lymph nodes were not as mature as those of the controls, and the number of secondary follicles was comparatively low. The spleen of prenatally treated animals had a rather embryonic character and contained few Malphigian corpuscles. However, there were essential differences between the corresponding organs of the test animals themselves, so that the histological picture did not permit of definite conclusions.

Mathematical Analysis of the Results

Comparing the numbers of animals per litter (approximate Poisson distribution) by means of Student's *t*-test after square-root transformation, it was found that cortisone treatment significantly reduced the number of littermate animals ($p < 5$ per cent). The difference amounted to nearly two animals (8.6 against 6.7).

Individual birth-weights were subjected to a two-stage analysis of variance. The variance component, indicative of the effect of treatment, significantly exceeded the fluctuations of body weights within litters, but was not essentially in excess of the maternal effect which proved significant ($p < 0.1$ per cent) itself.

It follows that the change in total litter weight, induced by the treatment, could also be considered significant statistically.

It was impossible to analyse the changes in average body weight per litter with reference to the entire curve. (Fig. 1 presents curves showing the average values for a test and a control group, which are typical of

the other pairs as well.) The question arose whether absolute or relative deviations should be examined, at what times, etc. The end of the 5th or 6th week (the 35th or 42nd day) seemed the most suitable for determining the effect of treatment because, in earlier stages the weight curves ran more or less close, or because inferior weights of the treated animals may have been due to their lower weights at birth. Our comparisons were based on an equal probability of the weight of the animals of any group being superior to those of the other ($p = q = 1/2$). Comparison of matched pairs of test and control groups always showed higher values for the controls although these litters were more populous.

Compared to all test groups there was only one outlier among the three surplus control groups: on the 35th day, body weight was lower than in any of the test groups, whereas on the 42nd day it was inferior to only one of the test figures. We therefore feel justified in accepting the combined results obtained in the corresponding groups, namely, that gain in body weight was significantly decreased by the effect of cortisone ($p \sim 1/2\,5$, i.—e. $p < 5$ per cent).

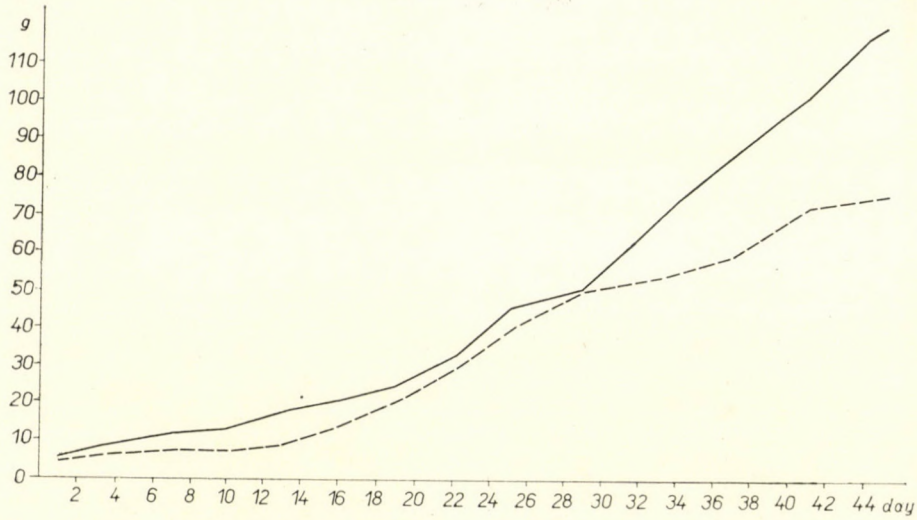

FIG. 1. Typical growth curves. Controls: ——— Tests: — — —

Percentage death rates, based on mortality up to the 45th day, were likewise analysed according to the above principle. Mortality among the test animals was significantly higher ($p < 5$ per cent). The difference was similar ($p < 5$ per cent) if we referred the death rates to the number of animals surviving until the third day; only one of the three surplus controls showed a value in excess of some test animals. As regards test animals, the number of deaths during the first

three days was significantly higher ($p < 5$ per cent), too. No difference was found between tests and controls in weight of the spleen on the 45th day ($p > 90$ per cent).

As to thymic weight, this was significantly lower in the offspring of cortisone-treated mothers ($p < 0.1$ per cent).

Absolute numerical values are listed in Table I.

placenta being impermeable to cortisone. This theory is, however, untenable since ANGERWALL and LUNDIN [1] have demonstrated the elevation of corticoid level in the foetuses of rats which had received cortisone during pregnancy.

Subsequent experiments [6, 10] confirmed the findings of FRASER et al. concerning palatoschisis in mice. Its incidence is, however, not the

TABLE I

Numerical results of the experiments

Statistical values	Number of litters		Birth weight		Weight of spleen		Weight of thymus		Percentage mortality	
	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
Number (n)	13	9	89	78	10	32	10	32	6*	8*
Average (*)	6.85	8.67	4.85	5.26	654	646	293	404	69.2	28.2
Deviation (s)	—	—	0.27		328	280	110	69	—	—
Significance (p%)	<5		<0.1		>90		<0.1		<5	

* Number of litters

DISCUSSION

According to a report of FRASER et al. [5], 2.5 mg doses of cortisone acetate administered to mice daily until the 11th to 14th day of pregnancy induced palatoschisis in 100 per cent of the foetuses. Cortisone has accordingly been classified as a teratogenic drug, although its teratogenic effect on human subjects has not been demonstrated reliably [11, 13]. Attempts at inducing deformities in rats by means of cortisone or prednisolone [1, 7] were unsuccessful. JOST [7] attributed this failure to the rat pla-

same in all mouse strains. While — according to FRASER's original report — cleft palate was registered in 100 per cent among members of strain A, the incidence amounted to 19 per cent in mice of the strain C57Bl.

It follows that, as regards teratogenesis, a drug may exert different effects in different species and even in different strains of the same species. Cortisone, as we have seen, has a 100 per cent effect in A mice and none in rats.

The very fact that cortisone is known as a strongly teratogenic drug

in certain cases, while — so far — it has not been proved to have such effect in rats, made it eminently suitable for our experiments. Its administration to pregnant rats was to show whether it would still remain harmless if the concept of teratogenesis was to cover a wider range.

There was one more reason for our choice of cortisone. According to certain experimental data, (i) cortisone, if administered to newborn rats, induces wasting disease, similar to that following thymectomy, which leads to marasmus and death [2, 4, 12]; (ii) cortisone, administered to pregnant rats, reduces the weight of the thymus and spleen of the offspring; (iii) the development of the rat thymus occurs between the 12th and 15th day following impregnation [9] so that cortisone administered at this time may influence all lymphatic organs and so the animal's development. On this basis it was justified to expect that — by observing the development of the offspring of mothers treated with cortisone during pregnancy, and by the choice of suitable parameters — we should be able to observe and register possible changes and anomalies.

It is clear from the present experiments that cortisone, administered during intrauterine life, may kill the foetus. This seemed to be proved by the significant difference (nearly two animals) between the respective numbers of littermates in the test and the control groups. Birth weight was, moreover, less in the litter of treated than in that of untreated mothers. Considering that death (absorption) of the foetus is one of the gravest teratological phenomena, there can be no doubt that cortisone has a teratogenic effect in the rat.

This effect does not, however, manifest itself in the form of deformities; therefore, teratogenesis may escape notice if individual newborn rats and not groups are compared. This applies to a certain extent to other phenomena as well. It is evident that the growth-inhibiting action of cortisone manifests itself most strikingly at the end of the 5th or 6th week, when the difference in the respective growth rates between tests and controls is already considerable, and when wasting already begins in the test animals. The importance of the experiments and of the relatively small but significant and persistent weight differences is borne out by the fact that the number of littermates was invariably smaller (and, hence, their conditions of nutrition were more favourable) in the test than in the control groups. Therefore, the test animals ought to have developed better than the controls so that their backwardness would have been still more striking if the respective numbers of littermates had been equal.

As regards mortality during the 45 days of observation, its rate amounted to 28.2 per cent among the controls against 69.2 per cent among the test animals. Since all other experimental conditions were identical in the two groups, increased mortality must have been due to the corti-

sone effect. The high mortality rate, like lagging in development, is a late manifestation of the drug's teratogenic action.

The two phenomena are reminiscent of the wasting disease [8, 9]. The microscopic picture seen in the present experiments was also suggestive of wasting disease, It should, of course, be remembered that histological information was scanty in the present study because only the tissues of animals were examined which had survived until the 45th day, i. e., of not more than 30.8 per cent of the total. It is known from MILLER's experiments [8] in which he induced wasting disease by thymectomy that not all members of a neonatally thymectomized litter develop the disease. We were presumably dealing with a similar phenomenon in the present case. This theory of the "wasting-disease mechanism", is supported by the fact that the thymus of the test animals, although these too had survived until the 45th day, weighed still less than the corresponding organ of equally old controls.

In conclusion, it may be stated that the teratogenic potency of a substance — drug or hormone — goes beyond the induction of malformations perceptible at birth. Teratogenic substances may induce submicroscopic, biochemical phenomena which retard postnatal growth and may at a later date even become fatal. This should serve as a warning, and it shows at the same time that the concept of drug-induced teratogenesis has to be extended and should be looked upon from a wider than a merely morphological angle.

Summary

Pregnant rats were treated with cortisone and the number of littermates, their body weight, development and the weight of their lymph organs have been studied. It has been shown that cortisone treatment destroys part of the foetuses, and that the surviving young animals are retarded in growth and development. The resistance of the litter of cortisone-treated animals was reduced and their mortality rate excessive. The weight of the thymus was significantly less than in the controls, a phenomenon suggesting the probability that cortisone acted by the wasting disease mechanism. It is suggested that teratological testing of pharmaceutical products should not exclusively be based on morphological evidence, since drugs may induce ultrastructural biochemical changes in the foetuses which manifest themselves at later postnatal times.

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