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THE TERATOGENIC INDUCTION OF HYPERTENSION *

By ARTHUR GROLLMAN and EVELYN F. GROLLMAN

(From the Department of Experimental Medicine, The University of Texas Southwestern Medical School, Dallas, Tex.)

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A condition resembling hypertensive disease in the human has been induced in animals by a number of procedures involving usually some manipulation of the kidney (1). However, the regular appearance spontaneously of a disorder comparable to essential hypertension of the human has not been accomplished in the laboratory.

Experimental mammalian teratology has attracted much interest recently as a means of explaining the occurrence of certain congenital disorders in the human. Previous work in this field has dealt exclusively with organic malformations. However, functional disorders resulting without obvious structural changes, but based nevertheless on some induced cytological or enzymatic disturbance, might also be expected to occur as a result of teratogenic influences. There is evidence to indicate that essential hypertension represents a congenital disturbance in renal function (1). Since a disorder resembling the spontaneously occurring disorder of the human can be induced in animals by various hormonal, dietary and other measures, these same procedures were applied during pregnancy and resulted in the appearance of hypertension in the offspring when the latter attained maturity.

METHODS

Piebald rats of the McCollum-Evans strain, reared in the laboratory, were injected with hormones and drugs or subjected to certain dietary regimens, as noted in Table I (column 1). The progesterone was a commercial preparation in oil containing 5 mg per ml; the aldosterone [1] was the *d,l*-21-monoacetate in 95 per cent ethanol (1 mg per ml), diluted with 10 vol of normal saline prior to injection; the cortisone was the commercially available aqueous suspension of the acetate ester; and the deoxycorticosterone was the commercially available aqueous repository suspension. Chlorothiazide was administered orally by mixing the powdered drug with the animal's food. The low potassium and choline-free diets were obtained commercially (Nutritional Biochemicals, Cleveland, Ohio). Ethanol was administered as a 5 per cent (by volume) solution in the drinking water. A high salt intake was given by substituting a 2 per cent sodium chloride solution for the animal's drinking water. All diets and fluids were supplied *ad libitum*.

Treatment was begun at various times before, at the beginning (as determined by the presence of a vaginal plug or of spermatozoa in the vagina), or during pregnancy, as indicated in column 2 of the table. After parturition, therapy was discontinued and the animal was returned to its normal diet (commercial rat chow and tap water). The young were weaned at 20 days of age and their blood pressures determined in the unanesthetized state at approximately monthly intervals, beginning at the age of 6 months, by the method of Williams, Harrison and Grollman (2).

The animals were followed for periods of up to 2 years, at which time their kidneys and hearts were weighed and examined microscopically at autopsy.

As controls, untreated animals of the colony of the same strain as the experimental group were used. Control and experimental animals received the same basic diet and salt intake, modified in the experimental group as indicated in the protocols. Each mother was used for only one procedure and a single litter. All animals were kept under the same environmental conditions and handled in an identical manner.

RESULTS

The results of the present study, which are summarized in Table I, show that the administration of certain hormones and dietary procedures listed in column 1 of the table, when given over certain periods of pregnancy, result in an elevation of blood pressure of the offspring to hypertensive levels. The blood pressures given in the table are averages of a series of at least 6 consecutive readings taken at daily intervals after a preliminary period of training to accustom the animals to handling. In the animals which became hyper-

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[1] Kindly supplied by Ciba Pharmaceutical Products, Inc., through the courtesy of Dr. R. T. Hill and the Endocrine Study Section of the National Institutes of Health.

TABLE I

Effect of various drugs and dietary procedures administered to pregnant rats on the blood pressure of their offspring at 1 year of age

Experimental procedure	Day of conception on which treatment was initiated*	No. of litters	No. of offspring surviving at 1 year	Systolic blood pressure		p Value of difference between experimental and control groups
				Range	Mean ± SD†	
				mm Hg		
Controls		10	88	98–128	114 ± 7.4	
Aldosterone (0.05 mg daily s.c.)	1	3	15	125–160	140 ± 9.5	<0.001
Cortisone (1 mg daily i.m.)	1	1	4	140–150	144 ± 4.8	<0.001
Deoxycorticosterone (1 mg daily i.m.)	4	2	12	120–155	142 ± 8.1	<0.001
	15	2	11	100–120	112 + 5.1	>0.3 < 0.4
Progesterone (1 mg daily i.m.)	1	3	15	130–155	142 ± 6.5	<0.001
Chlorothiazide (166 mg daily p.o.)	8	1	5	130–155	145 ± 9.3	<0.001
Chlorothiazide (166 mg daily p.o.) with high salt intake	15	2	10	130–170	147 ± 10.6	<0.001
Low potassium diet	−5	1	4	130–140	136 ± 4.8	<0.001
	1	1	3	135–160	144 ± 13.9	<0.001
	7	1	5	130–150	140 ± 7.1	<0.001
High NaCl intake	−7	4	29	135–160	148 ± 6.7	<0.001
	5	1	5	140–160	152 ± 7.6	<0.001
	14	2	12	120–135	127 ± 3.9	<0.001
Choline-free diet plus ethanol (5%)	10	1	5	135–155	150 ± 8.7	<0.001
Ethanol (5%)	−7	2	10	100–120	113 ± 8.6	>0.5 < 0.6
NaCl (1%) as drinking water	−7	3	16	110–130	116 ± 7.9	>0.3 < 0.4
Deoxycorticosterone (0.1 mg daily i.m.)	−7	2	8	112–132	119 ± 8.7	>0.4 < 0.5

* A minus sign indicates that treatment was begun on day indicated prior to conception.
† SD = standard deviation.

tensive, the blood pressures rose progressively, being only slightly elevated at 6 months of age, but rising thereafter at a steady rate to attain the values shown in Table I at the end of 1 year. The blood pressure continued to rise subsequently at a slow rate, reaching values of 150 to 180 mm at the end of the second year of life.

As noted in Table I, when deoxycorticosterone was administered in doses of 0.1 mg daily beginning a week prior to conception, or when the injection of a larger dose (1 mg) was not begun until the fifteenth day of pregnancy, no significant increase in blood pressure of the offspring resulted. Intermediate doses (not shown in the table) gave equivocal results. Likewise, the administration of smaller doses of cortisone (0.1 to 0.3 mg per day) or of aldosterone (0.01 mg per day) throughout pregnancy failed to affect the blood pressure of the offspring. Only the higher dosages apparently are capable of inducing the changes responsible for the development of hypertension. The administration of 5 per cent ethanol (without a simultaneously administered choline-free diet) or of 1 per cent salt as drinking water also does not result in a significant elevation of the blood pressure of the offspring (Table I). Other agents, such as testosterone, were also found to be either ineffective in this respect when used in small doses (0.01 mg per day intramuscularly), or to interfere with gestation when used in larger amounts. When larger doses of the drugs or dietary procedures, as listed in the table, were effective in inducing hypertension in the offspring, they often also inhibited conception or induced abortion if treatment was begun prior to or early in pregnancy. It is for this reason that the induction of hypertension, as shown in the table, was attained only when treatment was begun after pregnancy was well established.

The second column of the table shows the period during which the drugs or dietary procedures were applied, a minus sign indicating that treatment was begun prior to impregnation. The period during which treatment had to be carried

out to induce hypertension in the progeny varied. Thus, when hormones were injected, treatment had to be continued throughout pregnancy or begun (as in the case of deoxycorticosterone) not later than the fourth day after conception. When begun later (as indicated in the experiment cited in the table), no elevation in blood pressure ensued. On the other hand, a low potassium diet, a high sodium diet, or the administration of chlorothiazide, could be initiated after pregnancy was well under way and nevertheless induce an elevation in blood pressure. When chlorothiazide was combined with a high sodium chloride intake (to accelerate the loss of potassium), treatment begun as late as the fifteenth day of pregnancy resulted in the development of hypertension in the offspring at maturity. However, when the procedures were applied earlier in pregnancy, there was a more pronounced effect on the level of the blood pressure, as might be anticipated.

In no case was hypertension induced when the use of the drugs or procedures was terminated about midway during pregnancy (tenth to fifteenth day) even if initiated prior to conception. Apparently the resultant hypertension is a consequence of action during organogenesis rather than on the process of implantation or during the earliest stages of embryogenesis.

Examination of the tissues of the animals revealed cardiac hypertrophy, the average weight of the heart of the hypertensive animals per kilogram of body weight being 20 per cent or more greater than that of control animals. This was a consistent finding in all animals examined, as was also a slight arteriolar thickening comparable to that seen in early essential hypertension of the human. There was no obvious morphological injury to the kidney nor any evident excretory dysfunction; hence the present experiments may be considered as having produced a disorder analogous to that of essential hypertension in man.

In addition to the elevation in blood pressure, other effects of the experimental procedures were often apparent. Thus, abortion or resorption of the fetuses was a common occurrence, the 25 litters listed in Table I representing the results obtained from a total of over 100 trials. As noted in the table, the number of viable young reaching maturity was also less than in the control untreated animals. In some instances obvious defects in the young were evident. Thus, progeny from animals receiving progesterone manifested obvious edema of the face and paws. The young of rats receiving aldosterone included some which were runted, with heads enlarged out of proportion to the rest of the body. The mortality among such deformed animals was very high. When testosterone and ethionine in varying dosages were used, no viable young were obtained. The latter drug, however, when administered to the animals after weaning, resulted in the subsequent development of hypertension. Thus, in a series of 10 rats receiving 1 per cent ethionine in their diet for a period of 10 days after weaning at the age of 3 weeks, the blood pressures at the age of 1 year varied from 140 to 160 mm Hg (average 152) compared with 108 to 120 in a series of control animals of the same colony.

DISCUSSION

The results of the present study have several implications of clinical importance. In the first place, they represent an experimentally induced analog of essential hypertension of man. Although a related condition has been produced many times previously by various manipulations of the kidney or by subjecting animals to various drug or dietary procedures, the present experiments for the first time produce a more analogous disorder in the sense that the condition is first apparent in adult life as is the disorder in the human, and involves no surgical or other manipulation during postnatal life. Moreover, histological examination of the kidneys revealed no morphologically evident lesion, by the ordinary methods of examination, as is also the case in the human, nor any evidence of excretory renal insufficiency. As in the human disorder, hypertrophy of the left ventricle was the predominant pathological finding at autopsy, accompanied at late stages of the disorder by moderate arteriolar thickening.

Although essential hypertension in the human has a definite familial incidence and one can predict that a given patient with a family history of this disease has a 6 to 1 chance of suffering from essential hypertension (3), many instances occur in which no family history is obtainable. The present results show that environmental factors affecting the fetus prior to birth might readily ac-

count for many instances of spontaneously occurring essential hypertension in the human. The results of the present study demonstrate that many commonly used drugs and dietary procedures might induce the subsequent development of hypertension in the offspring. It has become apparent that the embryo is highly susceptible to many environmental factors which exert little or no detrimental influence on the maternal organism (4).

Previous studies on the experimental induction of teratogenic disorders have dealt with structural malformations evident at birth. The experiments reported here for the first time demonstrate that it is possible to induce by prenatal measures a disorder comparable to essential hypertension of the human, which in its early stages is not accompanied by any obvious morphologic disturbance. The results support the concept that alterations in the maternal organism may at times be responsible for the development of such overt disorders as hypertension or diabetes mellitus in the offspring.

Only relatively few specific environmental agents (ionizing radiation, rubella virus, toxoplasma, cytomegalic inclusion disease, acute folic acid deficiency, and synthetic progestogens) are known to be teratogenic to man. However, it is probable that many drugs and nutritional, hormonal, and other environmental influences may cause not only congenital malformations but also may be responsible for the development of such disorders as diabetes mellitus or essential hypertension, particularly in those predisposed by inheritance toward such disorders.

The present findings emphasize the potential harmfulness to the offspring of a variety of drugs and hormones used for protracted periods during pregnancy. Progestational hormones and natriuretic agents have been used as prophylactics against abortion and eclampsia, respectively. Of the agents used in the present study, intrauterine fetal death has been described in patients with toxemia taking chlorothiazide (5), and progesterone has induced the development of pseudohermaphroditism in the human (6), while cortisone (7) and metabolic procedures (8) are well known experimental teratogenic agents.

As regards the mechanism whereby the drugs and procedures used in the present study induced their teratogenic effects, it is assumed that these are a consequence of action on the kidney, which is probably more sensitive in its early stages of development than it is in the adult. As in the human disease, the disturbance is progressive and only makes itself evident at a later stage in life. All of the agents and procedures used in the present study, with the exception of chlorothiazide, when used under certain conditions, have been shown to induce hypertension in the rat (9–11). In the case of chlorothiazide, it is probable that its effects are due to potassium deficiency and are thus comparable to the results obtained by a potassium-deficient diet (12). In support of this conclusion is the observation that the addition of sodium chloride to the diet of animals treated with chlorothiazide did not interfere with its action in inducing hypertension but actually accentuated this effect, presumably by further accelerating the loss of potassium from the body.

SUMMARY

The offspring of rats subjected during pregnancy to various dietary procedures or treated with certain hormones or a natriuretic agent manifest a gradually increasing blood pressure which reaches hypertensive levels at maturity. The animals suffer from no renal excretory defect nor do they manifest any obvious lesions in the kidney histologically. The condition accordingly simulates closely the congenitally appearing essential hypertension of the human.

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