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Characteristics of Hypertensive Disease Evoked in Rats by Single or Multiple Injections of Polyvinyl Alcohol.* (28749)

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It has been shown that administration of certain polyvinyl alcohol (PVA) solutions to various animals causes uptake of the polymer by many tissues and organs(1-3). More recently it has been found that daily subcutaneous administration of PVA solutions to rats causes the development of hypertensive cardiovascular disease, macromolecular hypertension(4-7), which is aggravated by a high NaCl intake(5), and by unilateral nephrectomy(8).

No information is available to indicate whether such prolonged treatment is essential or whether the response might be elicited by small quantities of material, but requires a certain "latent period" to become manifest. Accordingly the present experiment was designed to determine whether hypertension could be induced by brief acute treatment, followed by a period during which its effects were assessed.

Materials and methods. Thirty-three young female rats of the Holtzman strain were unilaterally nephrectomized (day 1) and divided into 4 groups. Rats of Groups 1, 2 and 3 each received respectively 2.5, 5.0 and 7.5 ml of 5% PVA.† Since the material is more effective subcutaneously than intraperitoneally(8) the former route was employed. Animals of the 3 groups each received 2.5 ml on day 2, but those of Groups 2 and 3 received a second injection on day 6, and those of Group 3 yet a third injection on day 9. Animals of Group 4 served as noninjected controls. All animals received a 1% NaCl solution *ad lib* to drink and Purina Laboratory Chow. Blood pressures were measured periodically by a tail plethysmograph and values in excess of 150 mm Hg systolic were regarded as hypertensive.

On the 170th day of the experiment a microhematocrit and hemoglobin (Cyanmethemoglobin method) measurement and a serum protein determination (Goldberg Refractometer) were made on each animal from a sample of tail blood. The survivors were killed on the 171st day. Various tissues were placed in 10% neutral formalin for fixation. Organs to be weighed were dissected free of extraneous material, dried on filter paper and weighed on an analytical balance. Sections were stained with Congo red and with hematoxylin and phloxine.

Results. It was assumed that the group

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† Elvanol, grade 71-30, I. E. du Pont de Nemours & Co., Inc.

TABLE I. Hypertension in PVA-Treated and Control Rats.

	Data	Polyvinyl alcohol injected 2.5 ml	5.0 ml	7.5 ml	Controls
Day 23	Survivors	8	8	7	8
	Hypertensive	—	—	0	—
	B.P., mm Hg	—	—	123 ± 4*	—
Day 43	Survivors	8	8	7	8
	Hypertensive	2	5	5	0
	B.P., mm Hg	145 ± 9	159 ± 15	180 ± 14	121 ± 1
Day 71	Survivors	8	6	5	8
	Hypertensive	2	5	3	0
	B.P., mm Hg	153 ± 12	162 ± 7	183 ± 24	124 ± 2
Day 112	Survivors	8	5	2	8
	Hypertensive	5	4	0	0
	B.P., mm Hg	165 ± 16	162 ± 8	126 ± 5	130 ± 3
Day 158	Survivors	7	5	1	7
	Hypertensive	2	4	0	4
	B.P., mm Hg	140 ± 6	175 ± 15	121	148 ± 8
Day 169	Survivors	5	4	1	7
	Hypertensive	2	3	0	4
	B.P., mm Hg	152 ± 12	175 ± 20	122	160 ± 12

* Mean ± S.E. of mean.

given 3 injections would be most rapidly and severely affected, nonetheless on the 23rd day of the experiment none was hypertensive although this was well beyond the period of time required to elicit hypertension in rats given PVA at the rate of 1 ml/day subcutaneously. However by the 43rd day, 2 of this group had died (from hydronephrosis and probably not due to the PVA), and 5 of the remaining 7 were hypertensive. This represented the peak incidence in this group, for thereafter mortality continued until at the end of the experiment there remained only a single animal, which had been normotensive throughout. Four of these animals developed pressures in excess of 200 mm Hg. Two which died early in treatment developed peripheral edema and ascites.

In the group given 2 injections the peak incidence occurred somewhat later being reached by the 71st day. Mortality was less severe than among Group 3. Two had pressures greater than 200 mm Hg by the 43rd day and these failed to survive until another reading was made. Two others later developed pressures above 200 mm Hg, one of which lived until the experiment was terminated. One rat remained normotensive throughout.

In the group given a single injection, 2 animals had pressures of 180 mm Hg or higher by the 43rd day. These both went on to higher pressures and died intercurrently. No other rats developed pressures above 174 mm Hg, and although the overall incidence of hypertension (which reached its maximum on the 112th day) ultimately reached 63%, three animals remained normotensive throughout.

The experiment was continued until salt hypertension began to develop in controls. It was felt that no purpose could be served by continuance because of uncertainty as to whether additional hypertensives occurring in treated animals could be ascribed to delayed effects of PVA or merely to the cumulative effects of salt. Slightly more than half of the saline controls had become hypertensive by the time the experiment ended. It is of some interest that there were PVA treated rats which failed to develop hypertension in every group. After maximal incidence of hypertension developed in any treated group, the average blood pressure tended to fall thereafter as the most severely hypertensive animals succumbed and the surviving normotensives increased their proportional representation within the group.

Blood findings did not indicate an appreciable and consistent change in either hemo-

TABLE II. Principal Findings in PVA-Treated and Control Rats.

Data	Polyvinyl alcohol 2.5 ml	Polyvinyl alcohol 5.0 ml	Polyvinyl alcohol 7.5 ml	Controls
No. rats—initial	8	8	9	8
final	5	4	1	7
Body wt, g—initial	88 ± 2*	91 ± 1	90 ± 1	90 ± 2
final	292 ± 9	286 ±13	262 ±	291 ± 7
Hematocrit, %	47 ± 1	42 ± 2	37 ±	45 ± 2
Hemoglobin, g/100 ml	15.6 ± .3	14.0 ± .9	12.0 ±	14.0 ± .2
Serum protein, g/100 ml	7.8 ± .2	7.2 ± .1	7.8 ±	7.8 ± .2
Organ wt: Liver	5.26 ± .32	6.03 ± .64	6.98 ±	5.98 ± .29
Kidney, g/100 g	.99 ± .05	1.01 ± .01	1.17 ±	.96 ± .06
Heart	333 ±11	386 ±27	409 ±	367 ± 7
Spleen, mg/100 g	237 ±15	331 ±11	498 ±	296 ±22
Polyvinyl alcohol content:				
Kidney	++	++	+++	—
Heart	±	+	+	—
Liver	0	+	+	—
Spleen	+	+	++	—

* Mean ± S.E. of mean.

globin, hematocrit or serum protein concentration due to PVA treatment (Table II), in marked contrast to the considerable reduction in each which characterizes rats under daily chronic treatment.

Organ weights indicated that animals given increasing amounts of PVA tended to have progressive enlargement of the spleen, heart, liver and kidneys. The terminal occurrence of salt hypertension among controls, with resulting cardiac and renal enlargement, gave a high value for these organs. The presence of but a single survivor in Group 3 obviates a comparison of organ weights in this group with those of any other. Adrenal weights did not vary among the groups. The data are given in Table II.

Histologic findings. Congo red-stained sections revealed that almost 6 months after receiving polyvinyl alcohol the material was still present in various organs and tissues. In all animals the material could be found within glomerular foam cells, and to a lesser extent in the interstitial spaces of the medulla. Higher doses of PVA were associated with the highest concentrations. Animals which died intercurrently all had larger quantities within the kidneys than were found in the corresponding organs of full-term survivors. The material was also commonly found within the adrenal medulla, the spleen, heart, and liver. Again the quantities observed were generally proportional to the amount the animal had received. At the lowest dosage, among the 5 full-term animals, PVA was absent from all livers, all but one adrenal medulla, from 2 hearts and one spleen. All of these organs contained the material in rats of Groups 2 and 3. A subjective estimate of the comparative quantities visible in representative sections of various organs is given in Table II.

Vascular lesions typical of hypertensive disease and of long continued salt overdosage were found in the kidneys, hearts, occasionally livers and spleens, and often in the pancreatic and mesenteric vessels. Glomerulosclerosis, interstitial nephritis, arteriolar sclerosis, tubular atrophy and hyaline cast formation were common in the kidneys of hypertensive PVA-treated animals, and to a lesser extent, presumably because hypertension was of briefer duration, among hypertensive controls. Sclerosis of individual glomeruli, and often of several within a given section was also noted in each of the animals which had never shown elevated blood pressures at any time, both those which had received PVA and controls. Tubular atrophy and cast formation were associated. No extrarenal vascular lesions were observed in any of them.

Discussion. It is evident that hypertension

can be induced with rather small amounts of PVA, thus avoiding the necessity of daily injections, although onset is slower. Of the regimes used the one- and two-injection systems appeared to yield the best results, causing the same overall incidence of hypertension and survival rate. Maximum incidence of hypertension tended to occur earlier at the 2-dose level, and the blood pressure of hypertensives appeared to be somewhat greater. Three injections caused the most rapid evolution of hypertension and highest pressures, but mortality was quite high.

In contrast with the animals given daily injections(4-7), those in the present experiment had much less tissue infiltration of PVA. Furthermore with 2 exceptions in the 3-injection group that died early from acute overdosage effects, there was none of the edema and ascites so typical of animals receiving daily injections. The latter invariably develop severe hypoproteinemia and anemia, also absent in this experiment. These differences are doubtless due in part to the lesser degree of renal injury occasioned by the circumstances reported here.

Glomerular and tubular lesions in the control group were not restricted to hypertensive animals, but were found in those that had always been normotensive. The relative infrequency of blood pressure determinations makes it possible that they may have developed transient episodes of hypertension that were missed. They did not, however, have the cardiac hypertrophy of hypertensive controls or lesions outside of the kidney. It would thus seem that prolonged salt treatment may be capable of inducing glomerular injury, and thereby tubular damage, without hypertension. The same lesions in hypertensive animals were, however, much more numerous.

Polyvinyl alcohol hypertension is readily induced in rats given distilled water to drink instead of saline, but augmented salt intake aggravates the condition(5). At present we do not favor the view that PVA causes a renal injury which then sensitizes the animal to salt hypertension, although until PVA is given to rats on a sodium-free diet the matter cannot be considered as settled. The fact that several PVA-treated animals managed to escape both the hypertension caused by the polymer and that caused by long continued sodium excess is therefore interesting. If PVA merely sensitizes to salt hypertension then one may reasonably conclude that treated animals which did not become hypertensive were those with an inherent resistance to NaCl excess. On the other hand if the mechanism of the two types of hypertension is different, then it would appear that animals resistant to PVA hypertension are also resistant to the induction of salt hypertension.

Summary. Unilaterally nephrectomized rats on 1% NaCl drinking solution were given one, 2 or 3 subcutaneous injections of a 5% PVA solution. Hypertension subsequently developed in some, but not all of each group, higher doses resulting in greater incidence. In general larger quantities caused earlier onset of hypertension and higher levels of blood pressure. The lowest and intermediate doses caused less overall mortality, but hypertension tended to be earlier in onset and slightly more severe among those given the intermediate dose. Tissue examination almost 6 months after treatment revealed PVA in many organs, particularly in the kidneys. The efficacy of a single injection of PVA indicates that the procedure described constitutes a convenient method of inducing experimental hypertension in the rat.

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