mg/egg level. Smaller quantities produced some minor liver damage but no particular abnormality as was noted in the case of AGS. An inquiry is now being made into the effect of other hydrazides upon the chick embryo. In general, these are lethal in mg doses and produce minor liver damage at lower levels. In no instance have other hydrazides produced the extreme inhibition of the liver as reported here.

Summary. Aminoguanidine sulfate when injected into the yolk of a 4-day embryonated egg produced a severe inhibition of liver development of the chick embryo when the specimens were examined 10 days later. Apparently, the parenchymal cells were the sites of inhibition as the surviving tissue, in addition to being reduced in mass, was composed largely of connective tissue and cystic structures. Other organs and tissues of the specimens appeared to be relatively unaffected. The most effective sub-lethal dose at 4 days incubation was ineffective when administered at 9-10 days of incubation. Other hydrazides possess peculiar effects which bear no resemblance to that of AGS.

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Development of Hypertension and Cardiovascular-Renal Lesions During Adrenal Regeneration in the Rat.* (22028)

FLOYD R. SKELTON. (Introduced by D. J. Ingle.)

Department of Pathology, Medical College of Georgia, Augusta.

During a study of the effect of bilateral adrenalectomy on the production of hypertension and cardiovascular-renal lesions by methylandrostenediol in the rat it was observed that one control animal developed hypertension. Autopsy showed that the right adrenal of this rat had been completely removed but that a large regenerated adrenal cortex was present on the left side attached to the adrenalectomy scar. The possibility came to mind that the hypertension in this animal may have been the result of excessive hormonal secretion by the regenerating adrenal tissue. The following experiment was performed to test this hypothesis.

Methods. Twenty-four female Sprague-Dawley rats averaging 62 g body weight were unilaterally nephrectomized and given a diet of Purina Laboratory chow and 1% sodium chloride solution *ad libitum*. Twelve rats served as controls and 12 were unilaterally adrenalectomized with contra-lateral adrenal enucleation(1). This procedure consists of a small incision in capsule of the gland through which the bulk of glandular tissue is extruded by gentle application of pressure with curved forceps. The capsule and some adherent cells of the zona glomerulosa are

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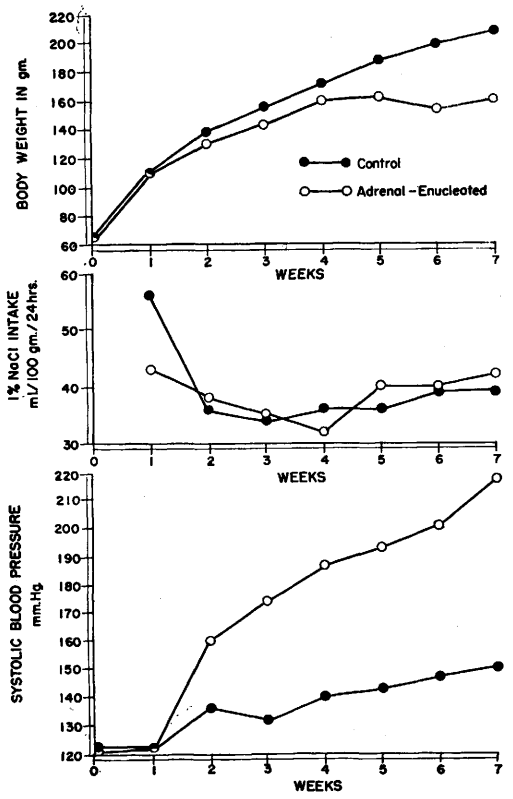

FIG. 1. Body wt, 1% sodium chloride consumption and systolic blood pressure of rats killed at 7 wk.

thus left *in situ*. Body weight, systolic blood pressure(2) and average 24-hour saline consumptions were determined weekly. Six rats in each group were killed at 7 weeks, the remaining 6 in each group at 14 weeks. At each time-interval blood was taken from the abdominal aorta and serum sodium and potassium determined on the Beckman DU flame photometer and chloride estimations made by the method of Schales and Schales(3). Organs were removed, weighed fresh and fixed in either Zenker-formal or 10% formalin. Paraffin sections were stained with hematoxylin and eosin, and frozen sections of adrenals were stained with Sudan IV.

Results. The adrenal-enucleated rats grew slightly more slowly than the controls and at no time did they consume significantly more saline solution than control rats (Fig. 1 and 2). Despite absence of an increased sodium chloride intake the adrenal-enucleated rats developed hypertension beginning from the second to fourth week (Fig. 1, 2). Systolic blood pressure of adrenal-enucleated rats killed at 7 weeks reached a terminal maximum of 217 $\pm$ 9 mm mercury as compared to terminal maximum of 151 $\pm$ 6 mm mercury in control rats. The systolic pressure of the adrenal-enucleated rats killed after 14 weeks levelled off at approximately 200 mm mercury at 9 weeks and had a terminal maximum of 197 $\pm$ 16 mm mercury as compared to 145 $\pm$ 7 mm mercury in control animals. In Tables I and II it can be seen that the serum sodium, potassium and chloride values in the adrenal-enucleated rats did not differ from the controls at either the 7- or 14-week interval.

At 7 weeks there was highly significant enlargement of the heart, kidney and brain in the adrenal-enucleated rats (Table I). The weight of the regenerated adrenal cortex of these rats did not differ from the combined weight of the adrenals in control animals. The

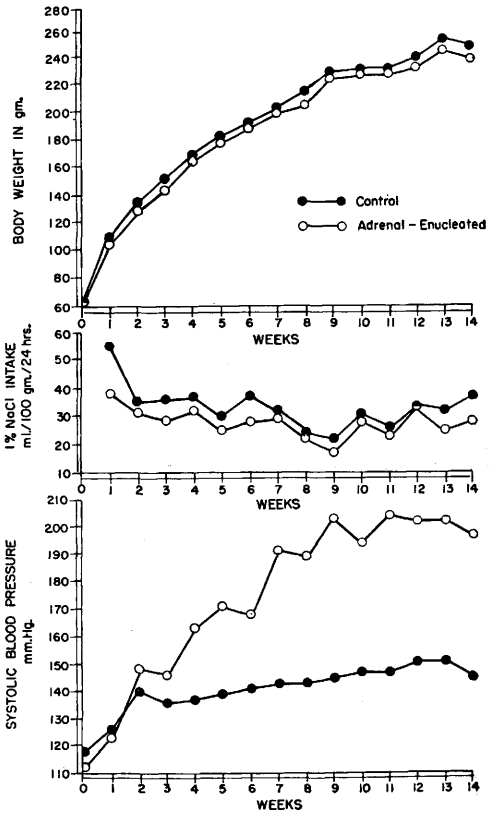

FIG. 2. Body wt, 1% sodium chloride consumption and systolic blood pressure of rats killed at 14 wk.

TABLE I. Organ Weights and Serum Electrolyte Values of Rats Killed at 7 Wk.

	No. of rats	Organ wt (mg/100 g body wt) Heart	Kidney	Brain	Adrenal(s)	Liver	Thymus	Spleen	Pituitary	Serum electrolytes (mEq/l) Na	K	Cl
Control	6	420 ± 9*	733 ± 36	823 ± 33	31.8 ± 1.0	4120 ± 220	177 ± 17	272 ± 6	4.6 ± .17	143.5 ± 0	4.42 ± .2	106.6 ± 3.4
Adrenal-enucleated	6	588 ± 31	1018 ± 108	1228 ± 34	29.0 ± .6	4987 ± 528	94 ± 22	316 ± 57	4.5 ± .4	145.2 ± 1	4.72 ± .3	110.0 ± 3.2

* Stand. error of the mean.

TABLE II. Organ Weights and Serum Electrolyte Values of Rats Killed at 14 Wk.

	No. of rats	Organ wt (mg/100 g body wt) Heart	Kidney	Brain	Adrenal(s)	Liver	Thymus	Spleen	Pituitary	Serum electrolytes (mEq/l) Na	K	Cl
Control	6	360 ± 13*	622 ± 44	716 ± 10	26.4 ± 1.2	3093 ± 109	107 ± 16	210 ± 18	4.6 ± .3	145.3 ± .9	3.9 ± .08	108.1 ± 1.8
Adrenal-enucleated	5†	442 ± 29	798 ± 47	753 ± 23	18.2 ± 1.6	3920 ± 249	92 ± 15	221 ± 28	5.6 ± .14	141.2 ±2.0	4.2 ± .3	108.7 ± .8

* Stand. error of the mean.
† One rat died before termination of the experiment.

thymus was significantly atrophic but nevertheless present in all animals bearing regenerating adrenals. No lesions were observed in control rats but in the adrenal-enucleated animals all 6 showed renal lesions, 5 showed cardiac lesions and 5 had periarteritis nodosa in either the pancreas or mesentery or both. Cerebral changes were observed in 5 adrenal-enucleated rats and consisted of focal cerebral hemorrhages and neuronal degeneration, edema and cyst formation with bulging of the cranial vault. Microscopically the renal and cardiac lesions and periarteritis nodosa were identical to those regularly produced by overdosage of desoxycorticosterone acetate(4, 5,6), and, except for the presence of periarteritis nodosa, are remarkably similar to the lesions of malignant hypertension in the human being.

In animals sacrificed after 14 weeks, the weights of kidney and heart of the adrenal-enucleated rats were significantly greater than in the controls (Table II) but less markedly than at 7 weeks. There was no cerebral enlargement or edema in the rats bearing regenerated adrenals. The weight of the regenerated adrenal gland was now significantly less than the combined weight of the 2 adrenals of the control animals, and the weight of the thymus of adrenal-enucleated rats was the same as that of controls. Although some control rats had developed scattered glomerular lesions similar to those in the enucleated rats, the lesions were more widespread and more severe in the latter animals. In general, however, the lesions in the adrenal-enucleated animals were less severe at 14 weeks than at 7 weeks. For example, mesenteric and pancreatic periarteritis nodosa was found in only one rat and no cerebral lesions were detected.

Discussion. Considerable evidence has been presented in support of the hypothesis (4) that derailment of the General-Adaptation-Syndrome involving hypersecretion of the adrenal cortex may lead to malignant hypertension and cardiovascular-renal lesions in the human being. This theory has been largely based on experiments in rats in which adrenocortical hyperfunction has been produced by exposure of the organism to nonspecific stress(7), the administration of either

anterior hypophyseal trophic substances(8), or corticoid hormones(9). Because the factor of non-specific stress has not seemed to play a prominent part in human hypertension and cardiovascular-renal disease many investigators have remained unconvinced of the importance of adrenocortical hyperfunction in their pathogenesis(10,11). Without assuming that there must be such a pathogenetic relationship, as outlined above, it is considered of some importance that the present experiment has demonstrated that hypertension and cardiovascular-renal lesions can be produced apparently as a result of hyperfunction of the adrenal cortex under conditions in which exogenous non-specific stress seems to play no part. The stimulus to both regeneration and to hyperfunction appears to lie entirely within the organism, and on the basis of extensive studies by Ingle(12) is assumed to be the lowered corticoid blood levels produced by the hemi-adrenalectomy and contra-lateral adrenal enucleation which in turn leads to increased secretion of ACTH by the anterior hypophysis.

Some thought concerning the type of secretion of the adrenal cortex under these conditions may be pertinent. Ingle has shown(12) that continuous administration of ACTH to adrenal-enucleated rats can cause such profound weight loss that the animal is brought to the point of death, presumably as a result of the greatly increased secretion of gluco-corticoids. Furthermore cortisone has been shown to produce hypertension and cardio-vascular-renal changes similar to those observed in the present experiment(13,14). However, this steroid produces increased consumption of sodium chloride drinking solution, profound thymic and splenic atrophy and does not cause the production of periarteritis nodosa but rather promotes the healing or prevents the development of such a lesion(13, 14). In this experiment no polydipsia occurred, thymic atrophy was never profound and present only at the 7-week interval, splenic atrophy did not occur and periarteritis was produced. Of further interest is the total absence of any significant changes in the serum electrolyte pattern.

All these observations suggest that the regenerating adrenal cortices were not secreting increased quantities of gluco-corticoids alone but rather that there was increased secretion of some mineralo-corticoid either alone or together with gluco-corticoids. Such a possibility is entirely compatible with the observations of Singer and Stack-Dunne(15), who demonstrated that the rat adrenal can respond to ACTH by liberation of both corticosterone and aldosterone into the adrenal vein. Furthermore it must be borne in mind that enucleation removes the zones which are presumed to secrete gluco-corticoids but leaves the zona glomerulosa which is considered to be the source of mineralo-corticoids, a situation which in itself might lead to imbalance of corticoid secretion. Regardless of how adrenocortical function may be altered in the present experiment the method makes possible future studies of the steroid secretory pattern of the adrenal cortex under conditions of increased function in the absence of superimposed exogenous stress and in which hypertension and vascular disease are a natural consequence.

Summary and conclusions. This report presents a simple technic for the production of a new type of experimental hormonal hypertension with cardiovascular-renal lesions in rats sensitized by unilateral nephrectomy and salt administration. It demonstrates that such hypertension and cardiovascular-renal lesions can be produced without polydipsia and excessive sodium consumption and without alteration in the serum levels of sodium, potassium or chloride. The results suggest that regenerating adrenal cortical tissue secretes increased quantities of cortical hormones and lend strong support to the hypothesis that increased adrenal cortical function is of fundamental importance in the pathogenesis of hypertension and cardiovascular-renal disease.

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Gonadotrophic Hormone Excretion of the Pregnant Monkey (*Macaca mulatta*).* (22029)

G. VAN WAGENEN AND MIRIAM E. SIMPSON.

Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Conn. and the Institute of Experimental Biology and Dept. of Anatomy, University of California, Berkeley.

The object of this study was to re-examine the reports that gonadotrophic hormone is excreted by the pregnant monkey for an exceedingly limited period of time. To determine simultaneously the type of gonadotrophin excreted by the monkey, the hypophysectomized rat was used to assay the urine. The first precise evidence for excretion of gonadotrophin by the pregnant monkey was furnished by Hamlett(1), who reported a positive Friedman reaction from urine collected between the 19th and 26th days. No urine collected after the 26th day of pregnancy gave a positive test. Delfs(2) found that gonadotrophic hormone could be detected (by the rat uterine-weight test) in the blood serum of pregnant monkeys as early as the 14th day of pregnancy; the amount reached a peak by the 20th to 25th day and the hormone disappeared by the 30th day. In both of these studies the time of gestation was accurately known from cycle and breeding dates. It appears that monkeys resemble man in that gonadotrophin is present in blood and urine during pregnancy, the hormone appearing in blood and urine soon after the establishment of the placenta; however, in the macaque, the hormone appears to persist for only a short period, whereas in apes (3-5) and man it is present to the end of pregnancy.

Methods. Female rhesus monkeys, *Macaca mulatta,* of the Yale obstetrics colony were placed with males at noon of the 11th day of the menstrual cycle. Each day thereafter a vaginal lavage was examined for the presence of sperm. If sperm were recorded on the 12th day and this was followed by pregnancy, day 12 was considered to be the day of conception. Urine was collected at representative periods from females in which the span of gestation was thus accurately known. Animals were placed in the metabolism cage at noon, for a 24-hour period, and the date of the 1st day identified that urine specimen. The urine drained through a paper filter and was collected under toluol. It was frozen immediately on termination of the collection period. To concentrate for injection, the urine was filtered, acidified to pH 5 and precipitated in 3 times its volume of ethanol. The precipitate was

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