17 exhibited positive direct antiglobulin reactions only with anti-γG globulin (Group 1) while 14 gave positive reactions with both anti-γG globulin and anti-complement serum (Group 2). As a preliminary test of Rh-related specificity, autoantibodies eluted from the RBC of patients in both groups were studied for their reactivity with Rh_{null} RBC. Twelve of 13 eluates showing negative or feeble reactions with Rh_{null} RBC were from patients in Group 1. Conversely, 13 of 18 eluates which gave reactions of equal intensity against Rh_{null} RBC and "normal" RBC were from patients in Group 2. The correlation of these apparent differences in antibody specificity with differences in the potential of γG autoantibodies for *in vivo* complement fixation has been discussed.

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Development of Chronic Low Plasma Volumes in Long-Term Adrenalectomized Dogs.* (32212)

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The plasma volume (PV) of intact and adequately maintained adrenalectomized dogs can be markedly reduced by various experimental means and held at the diminished values by feeding diets containing little sodium(1-5). The PV of intact animals, so treated, rapidly returns to normal upon resumption of the usual dietary regimen. Dogs lacking adrenals require glucocorticoids, *e.g.*, cortisone, dehydrohydrocortisone to accomplish the same result since mineralocorticoids such as aldosterone and desoxycorticosterone are not efficient for rapidly restoring the lowered PV and retaining it at normal levels(6). Long-term adrenalectomized dogs also appear to require periodic treatment with glucocorticoids in order to maintain normal plasma volumes. When they are kept for weeks or months on the usual mineralocorticoid and salt replacement therapy they eventually exhibit a gradual decline in PV which may fall to levels characteristic of animals suffering from severe adrenal insufficiency despite continuation of treatment. The ab-

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TABLE I. Effectiveness of i.v. Injection of 100 mg of the Solubilized Free Alcohol of Δ-1-Dehydrohydrocortisone for Raising the Chronic Low Plasma Volume of Long-Term Adrenalectomized Dogs Deprived of Food for 48 and Water for 24 Hours; Noneffect of Similar Injections upon the Same Animals when Exhibiting Normal Volumes and Subjected to Identical Treatment.

Therapy	Body wt (kg)	Blood urea N (mg %)	Blood sugar (mg %)	Hb (g %)	Hct (%)	Plasma volume (ml/kg)	Plasma volume % Change	Plasma electrolytes (mEq/l) Na	Cl	K
A. Chronic low volume dogs. Avg 11 animals.										
Chronic low PV	21.34	21.6	76	16.2	43.6	30.5		146	114	4.61
Glucocorticoid*	20.63	15.6	88	11.8	33.2	47.8	+56.7	146	112	3.61
B. Normovolemic dogs. Avg 10 animals.										
Normal PV	21.47	17.8	84	13.3	36.3	43.4		145	111	4.04
Glucocorticoid*	20.75	19.6	92	12.4	33.9	43.2	— .4	140	109	3.79

* Steroid administered i.v. in 4 divided doses during the last 24 hr of food and water deprivation. The final 25 mg injection was given at 9:30 a.m. and PV taken 1.5 hr later.
PV = plasma volume.

normally low PV may persist for long intervals without discernible untoward effect upon the animal's activity and vigor or significant alterations in the blood constituents.

Methods. The dogs were adult mongrels and had been without adrenals for approximately 3 years. During this time they were used frequently for studies of stress(7,8), adrenal insufficiency and action of vasodilator drugs upon the volume of fluid in circulation (9). Each cycle of induced circulatory failure and restoration to normal by i.v. injection of large amounts of glucocorticoids was routinely followed by 25-35 day intervals during which the dogs were maintained with PV at preadrenalectomy levels solely by daily i.m. injections of 1-2 mg of desoxycorticosterone in oil (DCA) plus 1-3 g NaCl in the food.

The dogs were trained to lie quiet and relaxed during the manipulations necessary for sampling and were fasted before testing. The PV method used was that recommended by Gregersen and Rawson(10) and Chien and Gregersen(11), using a standardized, weighed sample of the dye T 1824 and the dye-tinged plasma read on a Beckman DB Spectrophotometer at 620 mμ using 10 mm cuvettes.

Restoration of the chronic low PV to its original level was accomplished by: 1) rapid elevation within 24 hours following i.v. administration of 100 mg of the solubilized free alcohol of Δ-1-dehydrohydrocortisone given in 4 divided doses of 25 mg during the final 24 hours of a 48-hour food and 24-hour water deprivation period (Table I); 2) slower increase of PV in fed and watered dogs by daily i.m. doses of 50 mg of cortisone (Cortone, Merck), plus 2 mg DCA and 6 g NaCl in the ration (Table II). Restoration of the PV in the latter experiment was followed by withholding all therapy and permitting the animals to develop terminal insufficiency.

Results. Activity and vigor of the chronic low PV dogs remained undiminished even when the volume was reduced to the level commonly encountered when severe insufficiency symptoms are present. The food and water deprived animals reacted to i.v. injection of the glucocorticoid by sharp increase in PV within the 24-hour time limit set for the test. The magnitude of the rise was the largest recorded in this laboratory; increases in excess of 50% occurred (Table IA). Adrenalectomized dogs with PV at *preadrenalectomy* values, if fasted 48 hours and kept without water for 24 hours, do not reveal PV changes within 24 hours when injected i.v. with 100 mg of glucocorticoids (Table 1B), but readily do so if given 125 mg of the steroid in 5 divided doses(12). Before injection, the chronic low PV dogs showed an increased hemoconcentration over that of adrenalectomized normovolemic animals (Table IA and B), but except for the PV other differences were not marked. The weight and arterial pressure (not shown in Table I) did not undergo marked alteration after glucocorticoid treatment. Blood urea nitrogen tended to fall whereas blood sugar increased. Hemoglobin and hematocrit de-

TABLE II. Efficacy of i.m. Injections of Cortisone and Desoxycorticosterone plus Salt Supplements in Ration for Restoring to Normal the Chronic Low Plasma Volume of Long-Term Adrenalectomized Dogs Maintained Solely on Desoxycorticosterone and Salt and Subjected to Frequent Experimental Use; Terminal Insufficiency Following Withholding of Therapy.

Body wt (kg)	B. P. (mm Hg)	Blood urea N (mg %)	Blood sugar (mg %)	Hb (g %)	Hct (%)	Plasma volume (ml/kg)	Plasma volume % Change	Plasma electrolytes (mEq/l) Na	Cl	K
Average of 8 animals										
A. Normal PV.* Dogs receiving 2 mg DCA i.m. plus 1-3 g NaCl daily.										
20.60	123	17.7	80	15.23	39.3	45.2		148.1	112.9	3.44
B. Chronic low PV. Therapy same as in A.										
22.78	124	19.8	78	15.96	44.5	33.4	—26.1	148.2	113.8	4.53
C. PV 8 days after treatment with 50 mg cortisone, 2 mg DCA i.m. plus 6 g NaCl daily.										
22.17	123	14.0	85	15.06	40.6	45.1	+35.0	147.3	110.5	3.63
D. Severe insufficiency. Avg 11 days after withholding therapy.										
21.74	61	99.5	74	21.54	59.7	24.8	—45.0	124.4	92.2	6.65

* Normal PV data collected at various times during dogs' 3-yr stay in laboratory and given here for comparison only.

PV = plasma volume.

clined sharply as the volume rose. Plasma Na and Cl remained practically unchanged from preinjection levels but plasma K fell. Some of these changes were probably due to the increased volume of fluid in circulation following glucocorticoid injection. Pertinent data concerning the effect of i.m. injection of cortisone and DCA plus salt supplements upon the chronic low PV of the nonfasted adrenalectomized dogs are shown in Table IIB, C and D. DCA and salt feeding restored the PV within 8 days (average). Eleven days (average) after withholding all treatment, the animals developed gross signs of adrenal insufficiency and were sampled 12-24 hours before death occurred (Table IID).

Discussion. The low PV of adrenalectomized dogs suffering from severe insufficiency or experimentally induced shock is not easily restored to a normal level except by injections of large amounts of glucocorticoids. A gradual decline of PV invariably follows prolonged DCA and salt therapy but allows the animal sufficient time to make appropriate circulatory adjustments to the slowly decreasing amount of fluid in circulation. An acute loss of 56% of the plasma volume (Table I), presumably would result in circulatory collapse due to inadequate venous return to the heart, but if the blood-fluid loss occurred slowly and over considerable time, the loss probably would become chronic due to compensatory adjustments and hence could be tolerated.

The human geriatric patient and the chronically ill are said to exhibit chronic hypovolemia(13). One such circulatory adjustment to the lowered PV would require a gradually increasing rise in vasoconstrictive activity accompanying the decreasing circulating fluid volume. The chronic low PV tends to plateau when it falls to 30-33.4 ml/kg and may persist unchanged at this level for months. Any further drop initiates circulatory failure necessitating glucocorticoid administration. The data suggest that although C_{11} oxysteroids are vital for maintaining a normal PV and for revival of the animal from prostration and imminent death from circulatory collapse, it is obvious that mineralocorticoids and salt are effective for holding the chronic PV at the low plateau level during the long interim between glucocorticoid treatments. How this is accomplished is not clear. Terminal insufficiency quickly follows withholding DCA and salt therapy from these low PV dogs.

The greatly curtailed volume of circulating fluid in severe adrenal insufficiency and in shock induced by pentylenetetrazol (Metrazol), electroshock, etc., is probably due to sequestration of large quantities of blood fluid at the periphery of the vascular tree, perhaps owing to 1) development of an intense,

generalized vasoconstriction which may seriously interfere with free flow of peripheral blood(6-9) and 2) possible loss of vasodilating reactivity of the vasculature *per se* owing to lack of glucocorticoid. Large areas of the microcirculation appear to close, arterial pressure falls, hemoconcentration increases, muscular asthenia develops, the superficial veins lose turgor and may collapse from paucity of the contained fluid, the venous circulation becomes extremely sluggish with marked darkening of the viscous blood and increased difficulty is experienced in withdrawing samples from the large veins. Animals possessing functional adrenals quickly recover from these disabilities and have no need for additional therapy but dogs without these glands are unable to spontaneously increase the diminished quantity of actively circulating blood. Intravenous injections of glucocorticoids apparently function by somehow effecting release of the tightly constricted peripheral vasculature with consequent freeing of the restricted blood flow. The PV is then promptly restored to normal accompanied by return of the animal's usual activity and strength; this occurs in adrenalectomized dogs even when exogenous sources of food and water are withheld for 48-72 hours (12).

Summary. Adrenalectomized dogs maintained on mineralocorticoids and salt for long periods and used frequently for studies of insufficiency and effect of stress upon the circulation, eventually develop chronic low plasma volumes as depressed as those encountered in insufficiency, despite continuation of therapy. The low volume may persist for weeks, months, or until it is raised to normal by glucocorticoids. These animals exhibit normal activity and vigor with blood pressure, blood and plasma constituents little changed from those of normovolemic dogs. The chronic low plasma volume is assumed to be due in part to a generalized, gradual increase in vasoconstrictive activity, thereby reducing free blood flow at the vascular periphery and possibly also to loss of vasodilating reactivity of the peripheral vasculature *per se* owing to lack of glucocorticoids. These steroids appear active in opening up peripheral vascular channels and are necessary for reestablishing a normal volume of actively circulating fluid.

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