animals, but the effect is markedly exaggerated following Triton administration. Failure of the triglyceride secretory mechanism appears to be almost complete 2 hours after intoxication, which corresponds in time with attainment of the peak concentration of carbon tetrachloride in the liver(1). Failure of the hepatic secretory mechanism results in a dramatic fall in plasma triglycerides and a corresponding retention of triglycerides within the liver. The small rise of plasma triglycerides in the carbon tetrachloride poisoned rats injected with Triton could be due in part to a contribution from the gut(10).

The hypothesis in its present form has been restricted to the pathology of carbon tetrachloride poisoning. It has been tested only in regard to the action of this hepatotoxin. However, it appears self-evident to us that the hepatic triglyceride secretory mechanism must be complex and that it could fail as a result of a variety of pathological disturbances of the liver cells. It will therefore be most interesting to determine whether ethionine administration, choline deficiency, phosphorus poisoning, etc. likewise produce hepatic fat accumulation by destruction of or interference with the normal function of the hepatic triglyceride secretory mechanism. The elegant demonstration of this hepatic function by Byers and Friedman(10) has provided a simple method for determining the extent to which the hypothesis here presented is in fact a general one and not peculiar to carbon tetrachloride poisoning alone.

Summary. Intravenous administration of Triton to rats causes a marked elevation of plasma triglycerides. If the rats have been poisoned with carbon tetrachloride 2 hours previously, accumulation of triglycerides in the plasma following Triton administration is markedly reduced. The existence of an hepatic triglyceride secretory mechanism is inferred from the work of others, and it is postulated that carbon tetrachloride poisons this triglyceride secretory mechanism. Triglycerides, which would normally be secreted into plasma, are retained in the liver.

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Adrenal Ascorbic Acid and Corticosteroidogenesis Following Unilateral Adrenalectomy in the Rat.* (25924)

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In spite of widespread use of adrenal ascorbic acid concentration as parameter of adrenal cortical activity, little is known regarding its true relationship to corticosteroidogenesis. Since a sensitive method for deter-

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mination of corticosterone is now available, it appeared of interest to correlate changes in ascorbic acid and in adrenal and plasma corticosterone concentration, observed as ordinates of time following unilateral adrenalectomy in the rat.

Materials and methods. One hundred and seventeen adult male Sprague-Dawley rats, distributed into one group of controls and 16 of experimentals, were acclimatized for 7-10 days to laboratory conditions (constant lighting and temperature (25 ± 1°C); Purina Fox-Chow and water *ad lib.*) in batches of 6-12. In view of extreme sensitivity of corticosteroidogenic response to environmental change(1), they were caged singly and left undisturbed for 12 hours prior to experiment. Left adrenalectomy, used as stimulus and as means of securing an internal control for remaining gland, was performed by lumbar approach, under ether anesthesia. To prevent stress-induced elevation of its corticosterone concentration,† the control gland was removed within 90 seconds of anesthesia. The animals were killed by decapitation at progressively longer intervals‡ (1.5, 2, 4, 5, 10, 15 and 30 minutes; 1, 2, 4, 10, 24, 48, 72, 96 and 192 hours) from onset of etherization. Arteriovenous blood was collected from neck vessels into heparinized beakers for plasma corticosterone determination. Right adrenals of experimentals and both glands of controls were dissected free of adhering tissue and halved. The halves were weighed to nearest 0.05 mg and respectively extracted, following homogenization with rod and sand, in ethanol-saline for corticosterone, and 4% trichloroacetic acid for ascorbic acid determination. Adrenal and plasma corticosterone was determined by modification(2) of Silber's fluorometric technic(3); adrenal ascorbic acid, by modification(4) of Roe and Kuether's method (5). Results were expressed as percent changes from left adrenal levels for adrenal corticosterone and ascorbic acid, and from control baseline for plasma corticosterone.

TABLE I. Resting Levels of Adrenal Ascorbic Acid and Corticosterone in Control and Experimental Groups.

Group	Gland	Ascorbic acid, γ/100 mg fresh tissue	Corticosterone, γ/g fresh tissue
Controls (17)*	Left	462.2 ± 9.2†	8.4 ± 1.3
	Right	467.7 ±13.6	10.1 ± 1.3
Exp. (100)	Left	462.8 ± 7.5	8.6 ± .5

* No. of animals. † Stand. error.

Results. Resting levels. No significant difference was observed (Table I) between left and right adrenals of controls and left glands of experimentals, with regard to ascorbic acid and corticosterone concentrations. With due correction for residual fluorescence (6), mean plasma corticosterone level in controls was 3.3 ± 0.8 γ/100 ml.

Postoperative levels. A fall of adrenal ascorbic acid (Fig. 1A), detected within 4-5 minutes of surgical procedure and maximal (50%) after 1-2 hours, was followed by a

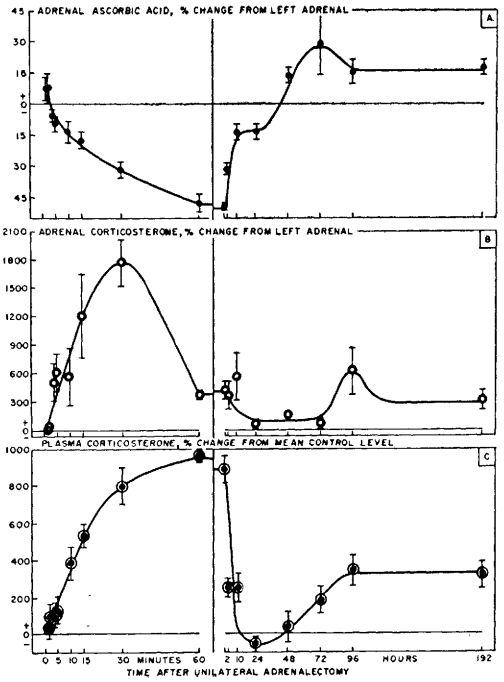

FIG. 1. Effects of unilateral adrenalectomy on ascorbic acid (A) and corticosterone (B) concentrations of remaining gland, and on plasma corticosterone level (C). Stand. errors are indicated by T-shaped bars.

† In few instances when left adrenal corticosterone concentration exceeded 20 γ/g, inadvertent stimulation was suspected and the data discarded.

‡ Antibiotics were administered on first postoperative day to animals maintained 48 hours or more after unilateral adrenalectomy.

rise interrupted at 10th hour, resumed at 24th, and levelling off above baseline after 3-4 days. Adrenal corticosterone response to the stimulus (Fig. 1B) was characterized by a 17-fold increase in concentration within 30 minutes, followed by return to baseline after 24 hours, and secondary rise after 3 days to moderately elevated values recorded on 4th and 8th day. The pattern evidenced by plasma corticosterone (Fig. 1C) differed by lower peak (ninefold increase) maintained one additional hour, and followed by regression to distinctly subnormal (40%) level after one day. Concentration increased, thereafter, to plateau of 300% observed between 4th and 8th day.

Discussion. The immediate postoperative changes in recorded parameters of adrenocortical activity are admittedly related to the release of ACTH triggered by surgical procedure. Hodges and Vernikos(7) showed that, under comparable conditions, level of ACTH in blood reaches its peak within 2½ minutes and is back to normal after 20. Since peak adrenal and plasma corticosterone values, and maximal ascorbic acid depletion were not recorded before 30 to 60 minutes postoperatively, it appears that the responses initiated by ACTH outlive its disappearance from blood. This persistent activation, noted by Brodish and Long(8) with respect to adrenal ascorbic acid, is ascribed by them to ACTH adsorbed by the gland and which, according to Sonenberg *et al.*(9), presents an exponential rate of disappearance. Duration and rate of corticosteroidogenesis could be likewise conditioned by amount of adsorbed ACTH and its biological half-life in the adrenal. This is consistent with the observed long-lasting effect of preincubation with ACTH on *in vitro* steroid production of rat adrenals subsequently washed and incubated in an ACTH-free medium(10).

No significance should be attached to the relatively smaller percentile increase of plasma than of adrenal corticosterone values, since baseline for plasma was obtained from intact donors, and should therefore be halved for strict comparison with values from unilaterally adrenalectomized animals.

The rise and fall of corticosterone in the gland are respectively indicative of a predominance of synthesis over release of hormone during first 30 minutes, and of release over synthesis from then on. It is interesting that ascorbic acid depletion appears more closely related in time to release than to synthesis of corticosterone, since maximal depletion does not correspond to peak of corticosterone concentration in the gland (30 minutes), but is achieved at the same time (60-120 minutes) as the peak of its concentration in plasma, when rate of release of the hormone presumably reaches equilibrium with rate of removal or inactivation. As a corollary, ascorbic acid repletion and fall of plasma corticosterone are initiated simultaneously, 2 hours postoperatively. This temporal relationship between initial changes in ascorbic acid concentration and release of corticosterone should, however, be interpreted with caution, in view of recorded instances of dissociation between the 2 phenomena(11,12). The relationship was not apparent, beyond the first postoperative day, when a secondary rise of plasma corticosterone paralleled continued repletion of adrenal ascorbic acid. Moreover, since the observed rise of plasma corticosterone concentration preceded by 2 days a corresponding change of its adrenal concentration, it would be difficult to dissociate the possible involvements, in early part of rise, of slower rate of inactivation and of increased rate of release of the hormone. The eventual rise of adrenal corticosterone concentration between third and fourth postoperative day is tentatively ascribed to the effect of transient hypocorticoidism recorded after 24 hours, on feedback-mediated release of ACTH.

Summary. From comparative study of changes in adrenal ascorbic acid and in adrenal and plasma corticosterone concentration induced by unilateral adrenalectomy in the rat, adrenal ascorbic acid depletion appears more closely related in time to release than to synthesis of corticosterone, since maximal depletion did not correspond to peak of corticosterone concentration in the gland (30 minutes postoperatively), but was achieved at the same time (60-120 minutes p.o.) as its

peak concentration in plasma, when rate of release of the hormone presumably reached equilibrium with its rate of removal or inactivation. In view of reported instances of dissociation between the 2 phenomena, the observed temporal relationship should be interpreted with caution.

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Actual Length of Diastasis for Regurgitation through Normal Mitral Valve; Cinefluorographic Demonstration. (25925)

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Regurgitation of blood can occur through a normal mitral valve. This has been demonstrated by: Hayden, *et al.*(1) utilizing isotope dilution; Friedman *et al.*(2) recording blood impedance changes after injecting sodium chloride; Wiggers(3) and Little(4), in measuring changes in pressure curves; and by Daley, *et al.*(5), using Evans blue in hearts with auricular fibrillation. Paul(6) has recorded this phenomenon by cinefluorography using opaque contrast in dogs with a slowed heart rate. The present study was undertaken as a further elaboration of Paul's work to determine the minimum length of diastasis to permit minimal regurgitation.

Method. Mongrel dogs, weighing 6.8-20.4 kg were anesthetized with sodium pentobarbital (35 mg/kg, *i.v.*). A No. 6 French whistle-tipped catheter was introduced *via* a thoracotomy incision into the left atrium through the auricular appendage and brought out through a stab wound in the chest. Pericardium and chest wall were closed, lung was fully expanded, and spontaneous respiration permitted to reappear. Two catheters (Nos. 6 F and 10 F) were then introduced into the left ventricle *via* the 2 common carotid arteries. The No. 6 F catheters in the left atrium and ventricle were attached to previously balanced strain gauges and pressures recorded simultaneously on a multichannel recorder. These pressures were subsequently used to measure period of diastasis (Fig. 1). After the heart was slowed by stimulation of the left vagus (tied off proximally) contrast material was rapidly injected into the left ventricle *via* the No. 10 F catheter. The opaque medium was visualized by means of a Philips fluoroscopic image intensifier(7,8) and

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