tion mechanism, the process of carcinogenesis, or both. However, it is to be emphasized that the data do not permit any conclusion with respect to the relationship between carcinogenesis and liver sulfhydryl concentration.

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Acute Choline Deficiency in Albino Rats: Vascular Contractility, Vascular Fragility, and Blood Pressure.* (23718)

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The rapidly developing and spectacular effects of choline deficiency in the weanling albino rat have been described(1-6). The most conspicuous feature of the deficiency syndrome is hemorrhagic degeneration of the kidneys, although hemorrhages are also observed in many other organs including eyes, heart, adrenals, liver and brain. The present study is concerned with an attempt to quantitate changes in vascular contractility and fragility during the syndrome, by technics to be described, and includes observations of blood pressure made during the course of the investigation.

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Methods. Composition of deficient diet was: casein 18%; lard 30%; cornstarch 30%; sugar 12%; agar 2%; salt mixture 4%; cod liver oil 4%; wheat germ oil 1%; salad oil 1%; and thiamine-8 mg, riboflavin-8 mg, pyridoxine-8 mg, calcium pantothenate-15 mg, nicotinic acid-15 mg, nicotinamide-15 mg/kilo of diet. A preliminary trial demonstrated that this diet produced typical findings of acute choline deficiency in albino weanlings, with mortality of 25, 62, or 40%, within 12 days. The supplemented diet was prepared by adding to the test diet choline chloride 500 mg/k, methionine 1 g/k, and inositol 100 mg/k. Twenty-five weanlings receiving this latter diet for 3 weeks showed a normal growth curve, and autopsies revealed no evidence of choline (or other) deficiency. 116 male weanling albino rats of Wistar

TABLE I. Vascular Contractility and Fragility.

Group	(A)	(B)	(C)	(D)	(E)
No. of animals	68	20	10	7	10
Age (days)	33	38	88	33	33
Avg wt (g)	47.3	71.1	238	45.3	55
Mortality (%)	14 (10)	0	0	0	0
Epinephrine threshold (million)	1:57	1:12	1:9	1:70	1:10
S.D. =	24.6	2.3	1.8	21.6	1.6
Electrical threshold (volts)	2.8	4.6	6.5	2.4	5
S.D. =	.69	2.6	2.7	2.4	2.6
Rupture threshold arterioles (volts)	8.3 (44%)	17 (15%)	†	†	†
Venules (volts)	9.1 (72%)	16 (30%)	20 (10%)	18 (10%)	20 (20%)
Capillaries (volts)	4.5 (37%)	7 (10%)	†	†	†
Voltage producing venular stasis & duration of stasis	7 12 min.	12 4 min.	15 5 min.	14 4 min.	12 5 min.

* Figure in parenthesis indicates % of animals in which rupture occurred.
† None produced.

strain were separated into 5 diet groups (Table I) as follows: Group A—*Choline deficient.* 68 animals (in groups of 6-10) were fed the deficient diet until signs of deficiency appeared (7-10 days), then were studied as described below. Survivors were given the fully supplemented diet an additional 45 days then the carotid blood pressure was measured by cannulation.

Group B—*Choline supplemented.* 20 animals (in groups of 2-4) were given the fully supplemented diet 7-10 days before examining capillary vessels, as below.

Group C—*Choline deficient hypertensive.* 11 animals. These were fed the deficient diet for 7-12 days until signs of deficiency appeared, then placed on fully supplemented diet for 45 days more to permit possible development of "choline deficiency hypertension" prior to study.

Group D — *Choline supplemented* — "starved." 7 animals. These were pair fed with 7 rats from the deficient group (A) for 7-12 days before examination, to observe possible vascular influences of the reduced dietary intake noted in deficient animals after 3-4 days.

Group E—*Standard laboratory pellet diet.* 10 animals were given the routine diet for 7-10 days prior to study of their peripheral vascular system, to rule out possible influences of the "synthetic" diet itself, even though fully supplemented.

Direct observations of the capillary bed were carried out on all 116 animals by exteriorizing the meso-appendix with Zweifach's method(7). After measuring the threshold responsiveness to topically applied epinephrine, relative reactivity to an electrical stimulus of known strength was determined with silver micro-electrodes and an electronic-type stimulator. By increasing the threshold voltage strength slowly, that necessary to produce rupture of the vessel wall in arterioles, capillaries, and venules could be established. It was also possible to discover the voltage levels necessary to produce intravascular "thrombosis" or stasis, within the venules.

Results. 1. *Vascular contractility.* The meso-appendiceal arterioles of the acutely choline deficient weanlings (Group A) showed a significantly increased reactivity to both topical epinephrine and electrical stimulation when compared with those on a fully supplemented diet (Group B) and the standard laboratory pellets (Group E), (Table I). This phenomenon cannot be attributed specifically to choline deficiency, however, for it was also observed in Group D receiving a complete diet, but in "starvation" amounts matched with the daily intakes of Group A. Olsen and Deane(8) have described hyperactivity of the zona fasciculata in weanlings during acute choline deficiency, which they attribute to the "alarming" stimulus of inanition. Deane and Shaw(9) had previously described simi-

lar alterations of the zona fasciculata with increased secretion of ketosteroids in rats on a moderate starvation regimen. Groups A and D therefore would be expected to have in common adrenal cortical hyperactivity, presumably due to an alarm response resulting from inanition. The question whether increased arteriolar responsiveness may be related to adrenal hyperfunction is one that is worth further study.

2. *Vascular fragility and venular stasis.* From the data in Table I, it is apparent that arterioles, venules, and capillaries of choline deficient rats are more susceptible to rupture by electrical stimulation than those of any other group, both with respect to the threshold stimulus required to produce rupture and to the frequency with which such damage can be produced. Hemorrhagic phenomena are widespread in acute choline deficiency, for hemorrhages can be noted in the eye, adrenals, heart, and liver, although with less frequency in these organs than in the kidney. During the minor manipulations of the abdominal organs incidental to the studies carried out, intestinal ecchymoses were common in the acutely deficient animals, and were not seen in the others, a gross corroboration of the data presented in the table. Increased fragility of these components of the capillary bed appears then to be specific result of acute choline deficiency in the young rat. Best(10) has recently demonstrated extensive deposition of fat droplets in the intima of vessels in acute choline deficiency. It may be that such a deposition of fat increases the fragility of these vessels. A similar explanation may be advanced for the marked tendency to venular thrombosis among the deficient group; the fat laden vessel wall resists injury poorly. In past investigations of acute choline deficiency in the young rat, most attention has centered upon the most prominent pathological feature, so-called hemorrhagic renal degeneration. The pathogenesis of this process has not yet been completely explained. Hartroft and Best(11,12) have stated that the initial lesion is accumulation of fat droplets in cells of the proximal convoluted tubules of the cortex. According to their observations, there follows swelling of the nephron, with obstruction of the cortical capillary plexus, which in turn leads to ischemia and hemorrhage. Christensen(13,14) has described "massive destruction of the vascular system in the peripheral zone of the cortex" during the acute deficiency stage. It seems likely that the loss of integrity of the vessel wall, particularly of the arterioles, manifested by increased vascular fragility and susceptibility to injury, may be considered an essential factor in the pathogenesis of renal degeneration in acute choline deficiency.

TABLE II. Blood Pressure Determinations in Rats after Acute Choline Deficiency.

	No. of animals	Avg B.P., mm Hg	S.D.
Group (1)—12-18 wk after initial study	18	154	22.7
Group (2)—6-12 wk	14	134	12.6
Control group—age, 16 wk	10	108	7.0

3. *Blood pressure determination.* Table II contains data which illustrate the upward trend of blood pressure with advancing age, in formerly acutely choline-deficient rats. These results are in agreement with the observations of Hartroft and Best. This steady rise in blood pressure is ascribed to the chronic nephropathy resulting from the acute renal injury, perhaps analogous to the progressively higher blood pressure in humans with chronic nephritis. In this study, 46% of the rats became hypertensive within 6 to 18 weeks. It would appear that this procedure could be utilized more widely as a method of producing experimental hypertension, inasmuch as it is very simple and attended by a negligible mortality.

Summary. 1. During the acute phase of choline deficiency in albino weanling rats, arterioles, venules and capillaries of the mesoappendix exhibit a significantly increased fragility, as measured by response to electrical stimulation, in comparison with control groups. There is also a definitely increased sensitivity of arterioles to epinephrine due to inanition. Venular stasis is more rapidly produced, and is of longer duration among choline-deficient animals than in controls. These vascular phenomena disappear upon addition

of choline to the deficient diet. 2. The hypothesis is advanced that these phenomena are reflections of an increased susceptibility to injury of the vessel wall, probably related to deposition of lipid, and that this condition may be an essential factor in the pathogenesis of hemorrhagic renal degeneration. 3. Blood pressure of formerly choline deficient rats tends to rise with time. It is suggested that this method of producing experimental hypertension be used more frequently, because of its simplicity and effectiveness.

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Enzymatic Defect in Metabolism of Bilirubin in Fetal and Newborn Rat. (23719)

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Hyperbilirubinemia and kernicterus can occur in the newborn in the absence of increased hemolysis of the erythrocytes(1-4). The incidence of this nonhemolytic hyperbilirubinemia is particularly high in the premature infant. A low rate of clearance of bilirubin in the liver due to some functional immaturity of that tissue may be responsible for the condition(5). Since bilirubin apparently cannot be cleared by the fetal liver, it has been suggested that this pigment may pass through the placenta and be carried to the mother's liver(6) or may be metabolized by the placental tissue itself(7). Bilirubin is excreted in bile as a conjugate with glucuronic acid (8,9). This conjugation takes place in the liver, to a lesser degree in the kidney(10), and possibly in the stomach and intestine(11). A scheme of the conjugation mechanism for glucuronides has been proposed(12): glucose-1-phosphate + uridine triphosphate ⟶ uridine diphosphoglucose $\xrightarrow{DPN}$ uridine diphosphoglucuronic acid (UDPGA) + aglycone ⟶ aglycone glucuronide. Although conjugation of *o*-aminophenol with glucuronic acid could be demonstrated in slices of adult rat liver(13,14), no appreciable conjugation was found in slices of fetal rat liver.

In a previous study using tissue homogenates, we demonstrated a defect in the conjugation of bilirubin in adult rats with constitutional nonhemolytic hyperbilirubinemia(15, 16). In the present study homogenates were again used to investigate the nature of the mechanism for synthesis of bilirubin glucuronide in tissue from prepartum and postpartum rats of various ages.

Methods. The homogenate system used was similar to that described previously(10). Forty micrograms of bilirubin suspended in an albumin solution were added to 100 mg of tissue homogenate in phosphate buffer. One ml of boiled extract of adult liver(17) (extract A) or of fetal liver from animals 5 to 7 days prepartum (extract B) was also added as a source of UDPGA. The mixture was incubated for 45 min. at 37°, then centrifuged in a Servall refrigerated centrifuge