insulin for the erythrocytes. By a number of technics, this change in blood cell-plasma partition was demonstrated to be attributable to an insulin-binding globulin induced only in insulin-treated subjects, irrespective of their diabetic status. These technics included paper strip chromato-electrophoresis, starch block electrophoresis, ultra-centrifugation of insulin I^{131}-plasma mixtures, precipitation of insulin antibody complexes with rabbit anti-human globulin serum, etc.(3,4).

By use of a relatively simple technic, we believe that we have shown the presence of an insulin-binding plasma factor in subjects who have received insulin. We have not attempted qualitatively to correlate this with determinations done by other technics. However, because this plasma binding factor was insulin-induced and, as others have found (3,4), did not quantitatively correlate with either dosage or duration of insulin therapy, this procedure appears to demonstrate the presence of the reported insulin-binding globulin. In these studies the reduced RBC uptake appeared between the third week and third month of the institution of insulin treatment; it persisted for longer than 2 months and, in one patient, for 4 years after insulin treatment had been discontinued. This time relationship is consistent with the appearance and disappearance of the insulin antibody as determined by other technics(3, 4).

It must be noted that the present method cannot distinguish between red blood cells and leucocytes. However, we have demonstrated that the leucocyte contribution to the blood cell uptake may be regarded as unimportant in this technic.

Summary. The capacity of RBC to take up radioactive insulin (I^{131}) from whole blood has been studied in blood samples from normal and diabetic subjects. Prior insulin therapy induces a marked reduction in RBC uptake. Dietary management and/or sulfonylurea therapy does not induce a reduced RBC uptake of radio-insulin. These findings are interpreted to represent a simple technic for demonstrating the presence of insulin antibodies.

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Received April 7, 1961. P.S.E.B.M., 1961, v107.

Effect of Triparanol (MER/29) on Corticosterone Secretion by Rat Adrenals.* (26621)

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Triparanol (MER/29) is used as an agent to lower serum cholesterol. Studies by Avigan, Steinberg and coworkers suggest that triparanol inhibits conversion of desmosterol (24-dehydrocholesterol) to cholesterol, the final step of cholesterol synthesis(1). There is also evidence which shows that the total sterol pool is significantly decreased during triparanol therapy(2). Blohm, Kariya and Laughlin(3) have demonstrated that feeding triparanol to rats results in a marked reduction of adrenal cholesterol. Since cholesterol is an important precursor of adrenocortical hormones, it was thought that triparanol feeding might also result in decreased steroid hormone synthesis by this gland. For this reason the present study was initiated.

* Supported by grants from Nat. Vitamin Foundation and Nat. Inst. of Arthritis and Metab. Dis.

Materials and methods. Young, male rats

TABLE I. Effect of Triparanol Feeding on Adrenal Function in Rats in Exp. I.

	Left adrenal wt (mg)	Adrenal cholesterol content (mg)	Adrenal vein plasma flow (ml/hr)	Plasma corticosterone (γ/ml)	Corticosterone secretion rate (γ/hr)
Triparanol treated rats (10)	24 ± .75*	2.55 ± .16	4.39 ± .45	12.8 ± .83	56.3 ± 6.4
Control rats (13)	20 ± .39	8.80 ±1.002	4.33 ± .44	17.5 ±1.39	71.4 ± 5.7

* Mean values ± stand. error of mean.

of the Holtzman strain were used. Rats were maintained on a diet of Purina laboratory chow and water *ad lib*. Triparanol was suspended in a carboxymethyl cellulose vehicle and fed to the animals by stomach tube.

Two experiments were conducted. In Experiment I, 15 rats, average initial weight 182 g, were fed 50 mg of triparanol per kg body weight for 13 days and 30 mg/kg for the next 12 days. The dose was reduced after 13 days because animals receiving triparanol were not gaining weight as rapidly as controls. Fifteen control rats, average initial weight 181 g, were given an equal volume of carboxymethyl cellulose for 25 days.

In Exp. II, 13 rats, average initial weight 192 g, were given 30 mg of triparanol per kg body weight by stomach tube daily for 35 days. Twelve control rats, average initial body weight 188 g, were given the vehicle alone.

Following the period of triparanol feeding, rats were anesthetized with pentobarbitol given intraperitoneally and adrenal vein blood was collected by the method of Longwell(4). Adrenal vein blood was collected for 2 consecutive 10 minute periods and since the volumes of the 2 samples were essentially the same, the samples were pooled, centrifuged and plasma taken for corticosterone analysis. Duplicate hematocrit determinations were done on blood from each rat. Plasma corticosterone was determined by the method of Silber *et al.*(5). When blood collections were complete, rats were sacrificed, the adrenals removed, dissected free of surrounding tissue and weighed. Total adrenal cholesterol was determined by the method of Knobil *et al.*(6).

Results. Exp. I is summarized in Table I. Triparanol feeding at a dose level of 30 mg/kg did not affect appetite or rate of growth of rats since at the end of the study treated animals weighed an average of 279.3 g while average weight of controls was 284.3 g. Adrenal vein plasma flows and left adrenal weights were also essentially the same in the 2 groups. Total cholesterol content of left adrenals from triparanol treated rats was decreased to 29% that of controls ($P = < .001$). Adrenal vein plasma corticosterone concentration was significantly lower in the treated group than in control rats, ($P = < 0.05$). Rate of corticosterone production was also depressed by triparanol; however, this reduction was not statistically significant since $P = > 0.05$.

In the second experiment (Table II) the period of triparanol feeding was extended by 10 days. Final body weight of treated rats averaged 348.5 g, while body weight of controls averaged 356.7 g. As observed in Exp. I, adrenal weight and adrenal vein plasma flow were not affected by triparanol feeding at this dose level. Adrenal cholesterol fell to 27% of control values in response to triparanol ($P = < 0.01$). Adrenal vein plasma corticosterone concentration and corticosterone secretion rate were both significantly depressed in the triparanol treated group ($P = < 0.05$).

Discussion. A considerable body of data has been accumulated through the use of adrenal slices, homogenates and perfusion technics which indicates that cholesterol is an important precursor of the adrenal steroid hormones(7,8). Other evidence, however, suggests that the adrenals can synthesize steroids from acetate without cholesterol being formed as an intermediate(9). The significance of this alternate pathway of steroid biosynthesis or confirmation of its occurrence has not been established.

Since cholesterol is an important precursor

TABLE II. Effect of Triparanol Feeding on Adrenal Function in Rats in Exp. II.

	Left adrenal wt (mg)	Adrenal cholesterol content (mg)	Adrenal vein plasma flow (ml/hr)	Plasma corticosterone (γ/ml)	Corticosterone secretion rate (γ/hr)
Triparanol treated rats (9)	25.2 ± 1.2 *	3.7 ± .27	4.92 ± .50	9.85 ± .91	45.8 ± 3.3
Control rats (9)	23.4 ± .95	13.8 ±1.6	5 07 ± .74	16.54 ±2.6	76.3 ±10.1

* Mean values ± stand. error of mean.

of adrenal steroids, it seems probable that any agent which inhibits synthesis of this sterol will result in diminished production of adrenal steroids. This hypothesis is borne out by results of the study reported here in which triparanol feeding caused a significant lowering of both adrenal cholesterol and the amount of corticosterone secreted by rats subjected to the stress of surgery and bleeding. Similarly, Melby, St. Cyr and Dale have demonstrated a 45% reduction in urinary excretion of 17-hydroxycorticosteroids in response to ACTH stimulation following a short period of triparanol feeding in humans(10). These investigators also found that urinary aldosterone and plasma cortisol levels were decreased in patients treated with this agent.

The finding that triparanol feeding results in a decrease in adrenal steroid production may have clinical significance in patients taking this compound. It seems conceivable that such an individual might develop acute adrenal insufficiency when exposed to the stress of major surgery or severe infection if not given prophylactic steroid therapy.

Summary. Triparanol feeding produces a significant reduction in the amount of corticosterone secreted by the adrenals of rats subjected to surgery and bleeding. It is postulated that this reduction is secondary to the marked decrease in adrenal cholesterol which results from triparanol therapy.

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Received April 7, 1961. P.S.E.B.M., 1961, v107

Potassium Content of Rat Diaphragms Following Hypophysectomy and Incubation with Growth Hormone.* (26622)

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The transport of α-aminoisobutyric acid (AIB) by isolated rat diaphragms is decreased following hypophysectomy and stimulated by addition of various species of growth hormone to the incubation medium(1,

* Supported by contract from Medical Research and Development Division, Office of Surgeon General, Dept. of the Army.