TABLE III. Effect of Pyridoxine Deficiency on Intravenous Mn^{54} Tissue Distribution.

Treatment	48 hr post inj interval		120 hr post inj interval	
	Pyridoxine deficient (6)	Pyridoxine treated (5)	Pyridoxine deficient (5)	Pyridoxine treated (4)
Brain	.312 ± .06*†	.283 ± .017	.367 ± .008†	.317 ± .022
Heart	.382 ± .03†	.477 ± .04	.244 ± .01	.289 ± .02
Liver	22.35 ± .3 †	24.74 ± .2	13.36 ±1.34 †	17.84 ± .72
Kidney	4.13 ± .24	3.54 ± .19	2.56 ± .07	2.52 ± .29
Spleen	.519 ± .04	.698 ± .02	.263 ± .02	.330 ± .03
Lung	.565 ± .02	.513 ± .07	.398 ± .03	.412 ± .05
Pancreas	—	—	2.46 ± .51	2.22 ± .45
G.I. tract	—	—	28.28 ±2.27 ‡	13.30 ±2.26

* Mean values and standard error of mean. Figures in parentheses indicate No. of rats used.
† Statistically significant difference from pyridoxine treated group, $P < .05$.
‡ Statistically significant difference from pyridoxine treated group, $P < .01$.

indicate that deficiency of pyridoxine is associated with increased uptake of the injected dose by brain and testicles, and with decreased uptake by liver and heart. No differences in Mn^{54} concentrations were observed in other tissues examined. The tracer content in the gastrointestinal tract and the feces of the pyridoxine deficient rats is significantly higher than in those of their respective controls. However, urinary excretion of radioactive manganese is reduced in pyridoxine deficient groups. These results suggest a role of pyridoxine in the regulation of manganese metabolism.

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Adipokinetic Effect of Intravenous Cortisol in Human Subjects.* (27891)

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The role of the adrenal cortex in regulation of lipid metabolism and transport in man is still uncertain(1). Elevation of serum lipids and O-lipoprotein has been observed in patients with Cushing's syndrome and in patients given cortisone therapy(2). No data are available relating adrenal corticosteroid administration to levels of serum fatty acids nor to lipid mobilization *in vivo* from adipose tissue in man(3). The present study was undertaken to investigate the acute effects of cortisol administration on levels of serum free fatty acids in healthy human subjects.

Experimental procedure. Materials and methods. Eighteen healthy volunteers were the subjects of the study. Eleven women,

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TABLE I. Levels of Serum Free Fatty Acids and Triglycerides in Subjects Given Cortisol.

Free fatty acids, μeq/l			Triglycerides, mg/100 ml		
Pre-injection	4 hr	6 hr	Pre-injection	4 hr	6 hr
368	669	869	130		102
688	1101	1239	114		60
468	635	769	76		54
447	809	998	99		75
602	852	1203	142		66
619	1239	1170	95		48
Mean ± S.D. 532 ± 158		1039 ± 193	109 ± 24		68 ± 19
585	1669				
702	1354				
430	1325		47	37	
511	1280		89	93	
Mean ± S.D. 542 ± 113 (total)	1093 ± 339				

p for free fatty acids 4 hr $<.001$
Idem. 6 hr $<.001$ All compared to pre-injection value using Student's t test.
p for triglycerides 6 hr $.001 < p < .01$

ages 19 to 60, and 7 men, ages 26 to 52, participated. All were ambulatory and fasted at least 12 hours prior to and during the test. They were divided into 2 groups. Ten subjects, 6 women and 4 men, were given 100 mg (2 ml) of hydrocortisone sodium succinate (Solu-cortef®, Upjohn) intravenously in less than one minute. Nine subjects received either 2 ml of 0.89% sodium chloride solution (4 women, 2 men) or 2 ml of cortisol vehicle† (2 men, 1 woman) intravenously in less than one minute. Blood samples were collected in cold, dry syringes prior to and 4 and 6 hours after injection. Blood specimens were immediately transferred to a chilled test tube, centrifuged in a refrigerated centrifuge at 0-4°C within one hour and the sera frozen immediately.

Free fatty acids were determined in all subjects in blood drawn 4 hours after the injection. In 6 of 10 patients given cortisol, levels of serum free fatty acid were measured 6 hours after injection. Of those given cortisol vehicle or saline, levels of serum free fatty acids were measured at 6 hours in 5 of the 9. Serum triglyceride levels were measured 4 hours after cortisol injection in 2 of the 10 subjects and 6 hours after injection in 6 others of the 10 subjects. Of the 9 subjects given cortisol vehicle or saline, levels of serum triglycerides were determined 4 hours after injection in 2 subjects, 6 hours after injection in 2 subjects and 4 and 6 hours after injection in 3 subjects. Serum free fatty acids were measured by the method of Dole and Meinertz(4) and triglycerides by a modification of the methods of Van Handel and Zilversmit(5) and Carlson and Wadström(6). Levels of serum total cholesterol (7) and of lipoproteins by paper electrophoresis(8) were obtained in all subjects prior to study.

Results. Mean pre-study levels of all serum lipids were within the normal range. Mean and range of values were: cholesterol, 240 mg per 100 ml (199 to 335 mg per 100 ml); triglycerides, 103 mg per 100 ml (41 to 460 mg per 100 ml); free fatty acids, 617 μeq per l (430 to 917 μeq per l); alpha-lipoprotein 22% of total (10 to 38%); beta-lipoprotein 78% of total (62 to 90%). Hypercholesteremia was noted in 1 subject, elevated triglycerides in 4 and abnormal lipoprotein distribution in 3.

All subjects who received cortisol showed a marked and significant increase in levels of serum free fatty acids measured 4 and 6 hours later ($p<.001$) (Table I). The mean level of free fatty acids was double the original fasting level at 4 and 6 hours. The levels of free fatty acids were comparable in the 4 and 6 hour serum samples. There was greater variation in the 4 hour response. There was a simultaneous significant fall in

† Vehicle, kindly suplied by Upjohn Co., Kalamazoo, Mich., contained 0.8 mg sodium biphosphate, 8.73 mg sodium phosphate exsiccated, 2.34 mg methylparaben, 0.27 mg propylparaben in water.

TABLE II. Levels of Serum Free Fatty Acids and Triglycerides in Subjects Given Saline or Vehicle.

Therapy	Free fatty acids, μeq/l			Triglycerides, mg/100 ml		
	Pre-injection	4 hr	6 hr	Pre-injection	4 hr	6 hr
Saline	447	860		92	74	
"	791	998		195	173	
Vehicle	610	774	844	69	83	75
Saline	482	551	895	174	176	143
"	826	895	1411	46	44	46
Mean ± S.D.				115 ± 65	110 ± 58	
Vehicle	895	810	756	62		55
"	722	980	586	57		49
Mean ± S.D.	*5 708 ± 165		898 ± 308	*5 82 ± 54		73 ± 40
Saline	603	798				
"	917	1454				
Mean ± S.D.	699 ± 172	902 ± 255				

p for free fatty acids 4 hr $.001 < p < .01$
p for free fatty acids 6 hr $.02 < p < .05$ All compared to pre-injection value using
p for triglycerides 4 hr $.05 < p < .1$ student's t test for small samples.
p for triglycerides 6 hr $.05 < p < .1$

p between control and cortisol free fatty acids at 4 hr $= .001 < p < .01$
Idem. at 6 hr $= .05 < p < 0.1$
p between control and cortisol triglycerides at 6 hr $= .001 < p < .01$

* No. of values from which mean was calculated.

the triglycerides of 38% at 6 hours ($.001 < p < .01$).

The control subjects who received either saline or cortisol vehicle showed a significant 31% rise in levels of serum free fatty acid at 4 hours after injection ($.001 < p < .01$) (Table II). The 27% rise in free fatty acids 6 hours after injection was of questionable statistical significance ($.02 < p < .05$). There was a greater divergence in values at 6 hours than was noted in the group administered cortisol. The small decrease in serum levels of triglycerides observed in control subjects at 4 and 6 hours (Table II) was not significant ($.05 < p < .1$). The rise in levels of free fatty acids at 4 hours in 10 subjects administered cortisol was significantly higher than the elevation following saline or vehicle injection in 9 subjects ($.001 < p < .01$). The increased rise in levels of free fatty acids at 6 hours comparing 6 subjects given cortisol to 5 control subjects was not of statistical significance ($.05 < p < .1$). The fall in serum triglyceride values 6 hours after injection of cortisol was significantly greater than the decrease observed in control subjects ($p < .01$).

Discussion. Circulating free fatty acids are released principally from adipose tissue(9) and are bound predominantly to albumin (10). The release of free fatty acids from adipose tissue is largely controlled by humoral factors. Epinephrine(11), norepinephrine(12) and adrenocorticotropin(13) induce release of free fatty acids from isolated adipose tissue. Intravascular administration of epinephrine or norepinephrine to intact human subjects and animals is followed by an acute rise in circulating free fatty acids(14).

Administration of human growth hormone acutely elevates serum levels of free fatty acid in intact human subjects(15). This action of growth hormone is noted in rats only in the presence of an intact adrenal gland (16). *In vivo* studies in man and dogs failed to show a clear effect of corticotropin administration on free fatty acids of plasma (15). Large doses of corticotropin given intravenously to adrenalectomized rats induced an increase in free fatty acids in adipose tissue and plasma. This was ascribed to activation of an adipose tissue lipase different from lipoprotein lipase. The adipokinetic mechanism was also different from that activated by growth hormone(17).

Jeanrenaud and Renold(18) demonstrated that cortisol and corticosterone added *in vitro* at concentrations of 30 μg per ml accelerated the release of fatty acids from rat adipose tissue. The exact nature of this action is not clearly understood but was attributed to facilitation of epinephrine response by the cortisone(19). Reshef and Shapiro(20) demonstrated that cortisone increased the sensitivity of adipose tissue to epinephrine without itself having any significant effect on the rate of free fatty acid release.

In the control studies with saline or vehicle the small rise in levels of serum free fatty acids at 4 hours could be attributed to endogenous release of epinephrine or norepinephrine. Increase in circulating free fatty acids was observed in human subjects with emotional arousal(21). The role of epinephrine was further supported by finding an increment in urinary excretion of the catechol amine during acute central nervous system arousal(22). In the present study serum free fatty acid levels were significantly increased 4 and 6 hours after the acute intravenous injection of cortisol. Thus it appears that the significantly greater rise in serum free fatty acids in subjects given cortisol 4 hours previously was attributable to the corticosteroid itself.

Of interest was the significant fall in serum levels of triglycerides noted six hours after administration of cortisol. Whether these acute changes can be reconciled with the increase in serum lipid levels induced by long-term administration of corticosteroids (2) is not clear. The latter studies in man, dog and rabbit did not include direct determination of serum levels of triglycerides but were primarily concerned with the development of hypercholesteremia. It has been postulated that accelerated triglyceride turnover accompanies increased metabolism of the precursors of free fatty acids(23). The net result might be either to increase or decrease serum triglyceride values.

Four hours after administration of 4 mg of human growth hormone by intramuscular injection to healthy human subjects a rise in free fatty acids of similar magnitude to that induced by cortisol was noted(15,24). Serum triglyceride values were unchanged at 4 hours(24). Measurement of serum levels of triglycerides 6 and 24 hours after a single injection of 4 mg of human growth hormone to healthy human subjects in this laboratory indicates no significant alterations. Other investigators have reported a decrease in serum triglycerides in 2 diabetic subjects given 12 and 16 mg daily of human growth hormone gel for 8 and 12 days(25). The varying effects on levels of triglycerides suggest that the adipokinetic mechanism induced by cortisol may be different from that due to growth hormone, with different sites of action of these 2 hormones.

The exact mechanism by which the rise in serum free fatty acids is brought about by cortisol is unknown. It may be due to a specific adipokinetic effect on adipose tissue activating an adipose tissue lipase, as has been suggested for corticotropin(17). The cortisol effect may be mediated through activation of the lipoprotein lipase system in plasma or in adipose tissue. Lipoprotein lipase was not detected in plasma following cortisol administration. Administration of cortisol in this laboratory was not followed by alteration in the lipoprotein lipase activation induced by heparin as measured by direct assay, free fatty acid levels and triglyceride values. In rabbits treated for 14 days with corticosteroids Day and Peters observed a clearing factor inhibitor using an artificial oil emulsion. Nevertheless in these animals after heparin there was both a rise in circulating free fatty acids and a fall in plasma turbidity indicating a dissociation of clearing from lipolysis. This inhibition was not found with intravenous administration of a single injection of hydrocortisone(26). Further studies of this enzyme system are under way.

Summary. 1. Intravenous injection of cortisol caused a significant rise in serum free fatty acids of 103% and a fall of 38% in serum triglycerides in healthy fasting human subjects. 2. Injection of either saline or cortisol vehicle induced a significantly lesser rise in serum free fatty acid levels and no significant change in serum triglyceride levels.

Addendum. Since the preparation of this

paper, Dreiling, D. A., Bierman, E. L., Debons, A. F., Elsbach, P. and Schwartz, I. L. (*Metabolism,* 1962, v11, 572) reported a biphasic response of levels of free fatty acids in normal subjects receiving hydrocortisone intravenously. They noted a fall of serum free fatty acids during the first hour, followed by a significant rise by the third hour. In 2 subjects in the present study who received cortisol, levels of serum free fatty acids rose 16% in one hour and in 4 of 5 control subjects who received either saline or vehicle there was a similar rise.

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Action of Purified Gonadotropins on Corpora Lutea in the Cyclic Mouse.* (27892)

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Isologous intraocular ovarian transplants restore normal estrous cycles in castrated female mice(1). Corpora lutea of each cycle initially show a distinct hyperemia that persists for only one or 2 days. This hyperemic phase is extended to approximately one week in pseudopregnancy, in the presence of an anterior pituitary transplant(2), and in lactation(3). Deciduomata can be evoked only when such corpora are present; the hyperemia appears to indicate function under the influence of pituitary luteotropin. The present report concerns the influence of exogenous FSH, LH and LTH on hyperemic corpora.

Material and methods. Female CE/BALB /c F1 hybrid mice, 2 to 3 months of age, in groups of 5 to 14 animals, were castrated.

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