TABLE III. Precipitation of Insulin-I^{131} from Incubated Mixtures of Insulin-I^{131} and Serum by NaCl-saturated 76% Ethanol Solution in 0.01 M KOH at −12°C.

Serum dilution	Type	% radioactivity precipitated	% radioactivity bound to Ab by chromato-electrophoresis	% Ab-bound insulin precipitated
Undil.	Control	7	None	
		15	"	
		6	"	
		6	"	
		6	"	
	Immune	51	82	62
		75	86	87
		63	71	89
		55	85	65
		90	91	90
		57	89	64
		75	75	100
		96	95	101
		89	90	99
		87	90	97
		85	90	95
		76	84	90
1:10	Resistant	86	90	91
"		70	79	89
"		87	90	96
1:50		78	90	87
1:10		91	90	101
1:50		80	80	100

paper chromato-electrophoresis. No more than 15% of free insulin was precipitated from control serums, while in 15 of 18 antiserums over 87% of the bound insulin was precipitated.

A large number of experiments was performed in which salt and ethanol concentrations were varied. In general, decrease in salt concentration or increase in alcohol concentration favored precipitation of free insulin with the serum proteins. With the modifications of temperature and pH described, the medium of Bates *et al.*(4,5) was found to be the most effective for the present purpose.

Summary. A rapid chemical method for detection of insulin-binding antibodies and separation of free and antibody-bound insulin in human antiserums is described. With NaCl-saturated 76% ethanol solution in 0.01 M KOH at −12°C to −10°C, 87% - 100% of antibody-bound insulin was precipitated from 15 of 18 insulin antiserums whereas 85% - 94% of unbound insulin in control serums remained in solution.

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Effect of Phentolamine (Regitine®) on Human Plasma FFA *in vivo*.* (25590)

ROBERT F. KLEIN AND MORTON D. BOGDONOFF
Dept. of Medicine, Duke University Medical Center, Durham, N. C.

Studies of plasma free fatty acid (FFA) levels in man and animals suggested that the sympathetic division of autonomic nervous system affects lipid metabolism(1). Administration of noradrenalin and adrenalin causes rapid rises in plasma FFA levels. Administration of an autonomic ganglion blocking

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TABLE I. Individual Response to Phentolamine Infusion FFA (μm/l) in 9 Subjects.

Dose, mg	Resting	During saline infusion	During phentolamine infusion	Time after end of phentolamine infusion, min. 10	20	30	40	50	60
5	480	498	—*	856	785	836	840	—	1157
5	384	351	—	465	408	454	—	370	385
15	507	429	661	692	672	—	—	—	621
"	611	813	721	1032	983	878	879	908	883
30	601	651	901	1079	1070	1082	—	1187	—
"	566	543	545	752	699	701	718	700	741
"	292	287	355	654	—	935	—	617	—
† "	754	664	1078	1158	1013	879	—	739	568
"	442	384	574	606	721	664	—	654	—

* — indicates no samples taken.

† Subject J.S. referred to in text.

agent lowers plasma FFA levels during the blockade(2). This study reports the effect of infusion of an adrenolytic agent (phentolamine, Regitine®) upon plasma FFA levels in man.

Methods. Eight hospitalized males and one volunteer male university student were studied. Their ages ranged from 19-56. All subjects were considered free of known metabolic abnormalities. None of the subjects was obese. They fasted 10-16 hours (overnight). All were previously informed as to the nature and general technic of the test. Subjects were studied in the supine position, with blood pressures and pulse rates taken by auscultation at 5-10 mintue intervals. Venous blood was drawn from a forearm or antecubital vein through an indwelling #18 Cournand needle. After a 20 minute rest period following needle placement, a saline infusion was given at 1-2 cc/min through a #18 Cournand needle in the opposite arm. By utilizing a 3-way stopcock, phentolamine infusion was started. Dosage of phentolamine was as follows: 5 mg in 2 subjects, 15 mg in 2 subjects and 30 mg in 5 subjects (the latter 2 doses given by a constant infusion pump at approximately one mg/min). Following the phentolamine infusion, blood samples were drawn at 10 minute intervals for determination of glucose (Somogyi-Nelson method) and FFA (Dole method).

Results. Table I lists FFA values obtained. Although there were no consistent changes during infusion, all subjects showed increased FFA levels following administration of phentolamine. These higher levels tended to fall after 20-30 minutes but in only one subject († in Table I) did they return to pre-injection levels at one hour after completion of infusion. Glucose values were essentially unchanged during and following phentolamine infusions.

All subjects showed a transient increase in pulse rate ranging from 4 to 26 beats/min during the first few minutes of infusion, with some subjects maintaining the increased pulse rate for duration of infusion. Blood pressure effects were as follows: A systolic and diastolic fall of 5-10 mm Hg occurred during the first 4-5 minutes of infusion, was followed by increase in systolic pressure, with diastolic pressure remaining depressed. The overall effect was an increase in pulse pressure of 10-20 mm Hg.

Discussion. The mechanism of this abrupt rise in plasma FFA levels following administration of an adrenergic blocking agent is unknown. The same phenomenon has been noted by Havel and Goldfien in the dog, using dibenamine(3). Benfey, Ledoux and Melville have demonstrated a marked increase in urinary catechol amines during development of adrenergic blockade by dibenamine(4). It is conceivable that the FFA response to phentolamine is one manifestation of a rebound transient increase in adrenergic activity.

Summary. 1) Nine human subjects were given phentolamine (Regitine®) infusions to observe the effect upon plasma FFA levels. 2) There was a consistent abrupt rise in venous FFA levels ranging from 100-500 μm/l without blood glucose changes. FFA values remained higher 1 hour after infusion. 3) The mechanism of this phentolamine effect on lipid metabolism is not known.

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Relationship Between Ribonuclease and Certain Anionic Polymers in Utilization of Mevalonic Acid by Liver Homogenates.* (25591)

LEMUEL D. WRIGHT, MARCIA LOEB AND ROSEMARIE HANSON
Graduate School of Nutrition, Cornell University, Ithaca, N. Y.

Preincubation of essentially whole homogenates of rat liver with crystalline ribonuclease (RNAse) abolishes capacity of these homogenates to convert mevalonic acid (MVA) into non-saponifiable material (NSF) or cholesterol (CHL). Control homogenates similarly preincubated ordinarily retain the capacity for biosynthesis(1). Inability of RNAse-treated homogenates to utilize MVA is associated with absence of ATP in these preparations. Homogenates preincubated with RNAse and an autoclaved liver extract (ALE) previously treated with an anion exchange resin to remove residual ATP maintain a level of ATP and convert MVA to NSF and CHL(2). Evidence has been presented for existence in these heated liver extracts of a factor concerned with maintenance of a functional level of ATP in homogenates, presumably involving oxidative phosphorylation(3,4), and a tentative mechanism for explaining the experimental data has been proposed(2,5,6). Allfrey and Mirsky have previously described a possibly somewhat similar system involving isolated thymus nuclei which when pre-treated with *deoxyribonuclease* lose capacity to convert amino acids into protein or purine and pyrimidine precursors into nucleic acids. The treated nuclei are devoid of ATP but the synthetic capacity is retained on preincubation with DNA or may be established by addition of DNA after inactivation. Activity is not confined to DNA, however, and RNA as well as a number of anionic polymers substitute for DNA(7,8). It is our purpose to point out that DNA and certain anionic polymers have an effect similar to the factor of ALE in the liver homogenate system and that these anionic polymers, but not ALE, are also inhibitors of RNAse. A conclusion that all anionic polymers necessarily function in the same way in maintenance of ATP levels in tissue may not be justified.

Methods and material. The experimental procedures involved in biosynthesis of NSF and CHL from MVA by rat liver homogenates have been described(9). In brief, cooled liver from young rats is homogenized quickly in "loose" homogenizer with nicotinamide-phosphate-magnesium buffer and centrifuged at slow speed to remove connective tissue. This essentially whole homogenate is dispensed into flasks containing ATP, DPN, succinate and RNAse where indicated, as well as various materials or combinations of materials to be studied with respect to biosynthesis of NSF. The flasks are aerated with a stream of oxygen, stoppered, and preincubated with agitation. Following preincubation MVA-2-C^{14} is added to each flask, aeration is repeated and flasks reincubated. Then contents of flasks are saponified, extracted with petroleum ether, extracts dried with sodium sulfate, filtered, evaporated, taken up in scintillation mixture and counted. The results given are in terms of cpm/incubation flask. Details of procedure are essentially as described previously. Despite use of nicotinamide as inhibitor of DPNase in the homogenizing buffer, DPN some-

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