Normal mean arterial pressure, based upon 44 Wistar origin females, age 2 to 5 mo., 3 successive measurements per rat on different days, averaged 129 mm Hg. Analysis of variance showed the rat to rat standard deviation (square root of rat component of variance) to be 7.7 mm Hg, and day to day standard deviation on a given rat to be 6.2 mm Hg(5). Most of our experience has been with Grollman type(6) renal hypertensive rats, in which B.P. as high as 300/200 have been recorded. We have considered rats as hypertensive when their mean B.P. was over 150 mm Hg, most of them ranging between 160 and 200 mm Hg.

Reliability of readings from the photoelectric tensometer(7) were studied by simultaneous recording of direct aortic B.P. Our tensometer, built within The Upjohn Co., recorded cuff pressure and light transmission through the foot simultaneously on a paper chart. Personal bias was minimized by accepting all clear-cut readings. A cuff as designed by Rosett and Handler was used(2). Five successive readings were made, the cuff removed and replaced in apparently the identical manner, and another 5 readings made; 4 such series an each of 10 different rats. Actual B.P. from the aorta varied only a few mm of Hg. Tensometer B.P. agreed well within the 5 replicate readings when the cuff was undisturbed (average range 25 mm Hg), but there were often large variations between averages of replicate series on the same rat (up to 70 mm Hg, average range of series means 40 mm Hg), which could be due only to cuff placement. That the presence of the tube in the aorta might have altered blood flow to the legs, and so caused such variations, seems doubtful since a similar series using a polyethylene tube tied into a carotid artery showed the same discrepancies. (Carotid cannulated preparations remained functional only a few days.) Within the limits imposed by cuff placement variation, tensometer and direct aortic systolic pressures agreed.

Summary. Method is described whereby polyethylene tubes, permanently implanted in aorta, may be used routinely for repeated direct measurement of arterial pressure in unanesthetized rats.

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Influence of Certain Basic Compounds on Conversion of Mevalonic Acid To Cholesterol by Liver Homogenates.* (25938)

LEMUEL D. WRIGHT AND ESTHER ENGLERT
Graduate School of Nutrition, Cornell University, Ithaca, N. Y.

Evidence has been presented that a polyanion is essential for maintenance of ATP levels in whole rat liver homogenates incubating aerobically(1). The "natural" polyanion in this system appears to be RNA(2) but other polyanions such as DNA, polyethylene sulfonate, and heparin may substitute for it(3). ATP levels are not maintained in the presence of the *polycation* protamine presumably because of formation of an anion-cation complex that renders the essential polyanion unavailable(4). Additional data are presented here concerning the specificity of the polycation as an inhibitor of the system essential for

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maintenance of ATP levels in aerobic homogenates.

Methods and materials. The biosynthetic experiments involved preincubation of 5 ml aliquots of a 200 xg supernatant fraction of rat liver prepared as previously described(1-4) with 5 ml amounts of solution containing 1 mg ATP, 10 mg DPN, 100 mg of sodium succinate, and various amounts of protamine or other compound studied. Each flask was aerated with a stream of oxygen and preincubated with agitation for 30 min at 37°. Following preincubation each flask was opened, 1 ml DL MVA-2-C^{14} solution added (500 γ, 50,000 cpm), the contents aerated, and the flasks then reincubated for an additional 3 hrs. After final incubation homogenates were saponified, extracted with petroleum ether, ether extracts dried with sodium sulfate, filtered, evaporated, taken up in scintillation mixture and counted. Reasons have been presented previously for a working hypothesis that conversion of mevalonic acid to nonsaponifiable material (NSF, largely cholesterol) under the experimental conditions described is a measure of ATP level existing at the time homogenates are supplemented with mevalonic acid(1). The triethylenemelamine (TEM) used was kindly made available by Dr. E. L. R. Stokstad, Lederle Laboratories, American Cyanamid Co. The trypsinized protamine was prepared by treating protamine in aqueous solution or in 0.1 M pH 7.0 phosphate buffer with 5% of its weight of crystalline trypsin for 18 hrs at 37° followed by heating at 100° for 15 min to inactivate trypsin. The other compounds studied were readily available commercial preparations.

Results. The data obtained are summarized in Table I. Inhibitory activity of protamine (MW 10,000 or less) in the system studied is markedly reduced by digestion of the protein with trypsin. Polylysine (MW 4,000) is even more inhibitory than protamine. Bovine serum albumin (MW 67,000) with an isoelectric point (4.8) considerably lower than that of protamine(10-12) is not inhibitory. Similarly, biosynthesis of NSF from mevalonic acid was not influenced by predigestion with such small molecular weight bases as putrescine, spermidine, spermine, arginine, or TEM.

TABLE I. Biosynthesis of NSF from Mevalonic Acid by Liver Homogenates as Influenced by Preincubation with Certain Basic Compounds.

Exp.	Material tested		cpm
1	None		8114
	1 mg	protamine	8060
	2		8065
	5		7936
	10		26
	20		25
	1	polylysine	7386
	2		9337
	5		32
	10		22
	20		33
2	None		7509
	5 mg	protamine	8314
	10		48
	20		34
	5	trypsinized protamine*	6694
	10		7264
	20		84
	5	bovine serum albumin	7050
	10		7274
	20		7763
3	None		5711
	4 mg	protamine	6052
	8		21
	12		22
	20		21
	4	trypsinized protamine†	6187
	8		8403
	12		5978
	20		3484
4	None		9503
	5 mg	protamine	9980
	10		31
	20		26
	5	putrescine	8660
	10		8362
	20		7328
	5	spermidine	9037
	10		8285
	20		7028
	5	spermine	9386
	10		9652
	20		9045
	5	arginine	9708
	10		9089
	20		9937
5	None		6037
	1 mg	TEM	5642
	2		5969
	5		5902
	10		5675
	20		5282

* Protamine trypsinized in water solution.
† Protamine trypsinized in 0.1 M pH 7.0 phosphate buffer.

Discussion. The results obtained are con-

sistent with an hypothesis that a polyanion essential for maintenance of ATP in aerobic homogenates of liver is made unavailable by high molecular weight polycations. The combination of large molecular weight and frequent positive charges seems essential for activity since (a) partial hydrolysis of protamine by trypsin lowers inhibitory activity, (b) bovine serum albumin exceeding protamine in molecular weight but containing few positive charges is not inhibitory, and (c) compounds such as putrescine, spermidine, spermine, and arginine comparable to protamine as bases but of relatively low molecular weight are not inhibitory. Apparently TEM under the conditions studied does not alkylate enough groups to reduce the effectiveness of the essential polyanion.

Summary. Conversion of mevalonic acid to non-saponifiable material by rat liver homogenates under conditions where conversion is a function of ATP level existing at time of mevalonic acid addition was inhibited markedly by preincubation with protamine or polylysine. Inhibition was less or absent following preincubation with trypsinized protamine, bovine serum albumin or a number of small molecular weight bases. The results are interpreted as indicating that a *combination* of high molecular weight and strong basicity are essential for inactivation of a polyanion previously shown to be essential for maintenance of ATP levels in liver homogenates.

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Investigation of Rose Bengal Conjugation.* (25939)

R. H. KUBIN,† G. M. GRODSKY AND J. V. CARBONE

Gastrointestinal Lab., San Francisco General Hospital, Metabolic Research in Arthritis and Allied Diseases, and Dept. of Medicine, University of California School of Medicine, San Francisco

Rose Bengal (tetraiodotetrachlorfluorescein) is one of the many dyes utilized in evaluation of liver function. Although uptake, storage and excretion of Rose Bengal I^{131} by the liver have been studied extensively, little is known of the metabolic processes involved. Clearance of Rose Bengal by the liver is impaired by concurrent administration of bromsulfalein (BSP)(1-4), and possibly by bilirubin(5). Recent studies have shown that hepatic clearance of BSP and of bilirubin involves conjugation with cysteine/glutathione (6,7) and glucuronic acid(8-10), respectively. Such observations suggest that these pigments may compete with Rose Bengal for initial uptake or subsequent metabolism by the liver and therefore that the competition may take place at a conjugation step. Attempts to demonstrate the presence of conjugates of Rose Bengal in biological fluids constitute the subject of this report.

Methods. Fresh human bile was obtained from 3 subjects with presumably normal liver function by means of T-tubes inserted in the common bile duct. After collection of control samples, 6-10 ml of Rose Bengal (Matheson Co.) (8.9 mg/ml) was administered intravenously; bile was collected at 30 min. intervals for 2 hr thereafter. For isotopic studies, 0.1 mC of Rose Bengal I^{131} (Robengatope, Squibb) was added to each dose before injection. Serum and urine samples were obtained from an additional subject with obstructive jaundice 45 min after injection of Rose Bengal. Control specimens were prepared by adding Rose Bengal *in vitro* to bile, serum and urine in concentrations approximating those

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† U.S.P.H.S. medical student part-time summer fellow, 1959.