those obtained by Kabat and Berg(7) in studying antigenicity of Dextran, another plasma expander which had been found clinically acceptable. As part of a larger study group Kabat tested 3 different samples of clinical dextran in 24 subjects. Of the 24, 9 showed positive skin tests after immunization with 7 of the 9 showing a 1+ or stronger reaction. A 1+ reaction was defined as a wheal of about 3-6 mm greater than that obtained in the same subject with the saline control with a surrounding erythema when read 15 to 20 minutes after the injection period.* Of these 9, 7 showed a rise of 2 μg or more of precipitable antibody nitrogen/ml of serum after immunization and 1 of the remaining 2 had more than 2 μg of precipitable antibody nitrogen/ml of serum prior to immunization. Maurer(10) obtained generally similar results in testing 4 samples of clinical dextran in 30 subjects, 8 of whom showed significant post-immunization antibody N rises with 6 of these 8 developing positive post-immunization skin tests.

It is difficult to make an exact comparison but insofar as the positive skin reactions in Kabat's study showed wheals as well as erythema plus other signs of reaction generally lasting 1 day or longer in the more severe cases (those showing a 2+ or 3+ reaction), as well as significantly positive serological tests, it seems that glutamyl peptide polymer compared favorably with dextran with respect to weakness of antigenicity in humans.

Many attempts to immunize rabbits and guinea pigs with our linear or polymerized glutamyl peptides have been negative.

Summary. 1) Antigenicity of a polymer of glutamyl peptide has been tested in 24 human volunteers. The polymer was of molecular weight and dimensions suitable for use as a plasma volume expander in humans. Skin tests, precipitin tests and complement fixation tests were performed on all subjects. 2) The results show 18 subjects with completely negative reactions by all 3 tests. There were 4 skin test reactions of dubious significance and one of real significance. There was one significantly positive serological reaction in a person showing a minimal skin reaction.

* Personal communication from Dr. Kabat.

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Effects of Reserpine on Cholesterol Levels in Cholesterol-Fed Rabbits.* (24346)

CESAR SOMOZA (Introduced by C. A. Krakower)
(With technical assistance of Harvey Locker)
Department of Pathology, University of Illinois College of Medicine, Chicago

It has been suspected that severe emotions and what is called "mental stress" might have some relation to elevations in blood cholesterol. Lyons(1) reported experiments in which serum cholesterol elevations were observed in cats when placed in cages surrounded by barking dogs. Selye(2) described a biphasic response of serum cholesterol in his

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adaptation syndrome. Recently Wertlake *et al.*(3) observed a significant increase in mean value of serum cholesterol in a group of healthy students under "mental stress" during examination week. It is not yet known what role, if any, the brain plays in serum cholesterol regulation. It has been shown however that a significant decrease in mean serum cholesterol is exhibited by cholesterol-fed rabbits following chlorpromazine administration(4,5) and that this drug induces a sharp fall in blood lipids in hyperlipemic man(6). It therefore appeared possible that reserpine might also have some action on cholesterol levels of cholesterol-fed rabbits.

Method. Thirty-four male albino rabbits, weighing an average of 2.823 kg, were divided into 3 groups: Group I, fed a stock diet containing 0.2% cholesterol, were injected 3 times weekly with normal saline. Group II, fed the same diet, received 0.04 mg/kg reserpine† IM 3 times weekly. Group III, fed stock diet without added cholesterol, received similar reserpine injections. Each rabbit of Groups I and II received daily 100 g of the cholesterol food. Food consumption was recorded. Each kg of cholesterol food was made separately and kept apart for each rabbit. Rabbits were weighed and about 6 cc blood withdrawn from ear vein at beginning of experiment and at end of third, fourth and sixth week. Total serum cholesterol determinations were performed by method of Abell, Levy, Brodie and Kendall(7). Feces of 10 rabbits of Groups I and II were collected during last 5 days of experiment. To exclude irregularities in physical distribution of cholesterol of stock diet during this last 5-day period, each rabbit daily received and consumed a separately prepared mixture of 100 g of the diet. Feces collected on each of days 2-5 were dried to constant weight, pulverized and sifted. Total cholesterol was determined on 3-4, one g samples from each specimen. Feces, with 10% alcoholic solution of KOH, were placed in boiling water bath for 3 hours. Cholesterol was extracted with 4, 50 ml portions of ether, washed and filtered. After evaporation, the sediment was treated as for serum cholesterol determination. Animals were killed at end of sixth week and a complete gross and microscopic autopsy performed. The aortas were thoroughly washed with physiological saline solution and, after brief fixation in 10% formalin, stripped of adventitia and stained with Sudan IV. Sections of liver, spleen and adrenals were also stained for lipids.

Results. During the experiment, 6 rabbits were found dead: 3 from Group I, 2 from Group II, and 1 from Group III. Of the remainder, 12 were cholesterol-fed controls (Group I), 10 were cholesterol rabbits treated with reserpine (Group II) and 7 were reserpine controls (Group III). No clinical side effects due to reserpine were observed.

Autopsy findings. No difference in weight of organs was observed between Groups I and II except for the adrenals. Mean weight of both adrenals in Group I was 540 mg; Group II, 420 mg; Group III, 330 mg. This difference is not significant. Amounts of cholesterol added to the diet had been reduced to a minimum to prevent overloading of rabbit's weak mechanism for cholesterol disposal. No considerable deposits of lipid, therefore, were expected to be found in the organs. Sections of liver stained with Sudan IV failed to show any sudanophilic material except in 1 case, a cholesterol control. Small amounts of sudanophilic lipid material were found in 4 spleens, 3 cholesterol controls and 1 animal on cholesterol diet and reserpine. Adrenals of cholesterol controls also showed larger deposits of sudanophilic material than those of Group II. After grading, the Sudan IV-stained aortas were divided into 3 groups. One group, containing moderate amounts of sudanophilic material, included 3 animals on cholesterol and 1 animal on cholesterol and reserpine. Another group contained 4 aortas completely devoid of sudanophilic material. This included 1 cholesterol control and 3 animals on reserpine and cholesterol. The third group included the remaining aortas in which content of lipid material was too small for accurate grading.

Hematoxylin and eosin sections taken from different organs showed no significant pathology with the exception of kidneys of 5 rab-

† Generously supplied by Ciba Pharmaceutical Products.

TABLE I. Total Serum Cholesterol in Reserpine-Treated and Cholesterol-Fed Rabbits.

	Group	No. animals	Total serum cholesterol, mean, mg/100 ml Wks of exp. 0	3	4	6
Cholesterol only	I	12	75 ± 27*	221 ± 98	236 ± 114	357 ± 189
" & reserpine	II	10	70 ± 25	159 ± 74	150 ± 82	199 ± 95
Reserpine only	III	7	72 ± 27	57 ± 18		81 ± 39
Significance of differences between Groups I and II (P values)				.1 < P < .2	.05 < P < .1	P = .02

* S.D.

bits: 3 rabbits on cholesterol diet treated with reserpine, and 2 reserpine controls. Some of these kidneys were paler than those of controls and, on section, showed a few minute yellow or red spots scattered over the medulla and cortex. Microscopic examination revealed discrete, well delimited lesions which seemed to affect only the tubules of the cortex and medulla. Some tubules in these areas were considerably dilated and others were collapsed. Interstitial tissues were extensively infiltrated with inflammatory cells. The type of inflammatory cell varied in different lesions, suggesting different stages of evolution. In some, infiltration was made up mainly of eosinophils and neutrophils. Minimal amounts of tissue debris were also seen. In other examples, infiltration was of a more chronic type and the predominant cells were lymphocytes and plasma cells. In an occasional case, no inflammation was seen but focal areas of fibrosis, possibly representing healed lesions. All of these lesions were observed only in animals of the 2 groups treated with reserpine.

Food consumption and body weight. There was little difference in food consumption between Groups I and II, and in gain in weight for all 3 groups.

Feces chemistry. There was no significant difference between values of cholesterol recovered from feces of rabbits of Groups I and II. Mean total cholesterol value/day was 150 mg in reserpine-treated rabbits and 130 mg in cholesterol controls.

Blood chemistry. Cholesterol levels rose progressively in all cholesterol-fed rabbits; however, after the third week of experiment, cholesterol levels in animals treated with reserpine increased at a much lower rate than in cholesterol-fed controls (Table I). At the end of experiment, mean value of serum cholesterol of reserpine-treated rabbits was considerably lower than that of cholesterol controls. Reserpine-treated rabbits on normal diet did not show significant variation of serum cholesterol level.

Discussion. The inhibitory effect of reserpine on hypercholesterolemia in cholesterol-fed rabbits seems unrelated to a deficient absorption of cholesterol in the intestine. No explanation can be advanced as to whether there is an increase in cholesterol catabolism or a decrease in formation of endogenous cholesterol. Possibly, the hypocholesterolemic effect might be mediated through the serotonin effect of reserpine. No studies, however, are available on effects of serotonin on serum cholesterol levels. The kidney lesion is similar to that seen following serotonin injections in the rat(8) and thus may be related to the altered serotonin levels reported by Shore *et al.*(9) following injections of reserpine. The scattered distribution and good delimitation of these lesions are suggestive of an ischemic mechanism.

Summary. Rabbits given injections of reserpine and fed a high cholesterol diet showed significantly lower mean serum total cholesterol levels and less lipid deposition in aorta, liver, spleen and adrenals than non-treated animals on the same diet. No variation was observed in rabbits treated with reserpine on a stock diet. Some rabbits treated with reserpine showed a kidney lesion similar to that described in rats treated with serotonin.

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Mitosis Stimulating Factor in Serum of Unilaterally Nephrectomized Rats.* (24347)

KAZUO OGAWA† AND WIKTOR W. NOWINSKI

Tissue Metabolism and Tissue Culture Lab., University of Texas, Medical Branch, Galveston

Glinos and Gey found that serum from partially hepatectomized rat has growth-promoting effects on primary liver explants and on strain of fibroblasts cultured *in vitro* with respect to initiation of outgrowth, organization and morphology of colonies, and duration of growth period(1). In addition, Friedrich-Freska and Zaki injected a rat with rat serum obtained 2 days after partial hepatectomy and found that the number of mitoses in liver increased 20 times whereas normal serum caused only a slight increase. The effect of serum was specific for liver and due to non-dialyzable protein (albumin or globulin) fraction(2). Teir and his co-workers investigated a growth-stimulating factor in liver homogenates from newborn or partially hepatectomized rats. When such homogenates are injected intraperitoneally into intact rats, the number of mitoses in liver of normal rat increases(3). They suggested existence of a local growth factor in addition to the general systemic growth regulation. In the present investigation, an attempt was made to demonstrate the presence of a growth-promoting factor in serum of unilaterally nephrectomized rat with respect to mitotic activity.

Methods and materials. Outer medullas of kidneys from Holtzman strain albino male rats, weighing 60 to 80 g, were cut in fragments approximately 1 mm square and explanted in plasma clot on 12 × 50 mm cover glass No. 1. The clot consisted of equal parts of heparinized rooster plasma and embryonic extract from chicken incubated for 8 days. Three explants were placed on each cover glass, and after clot had become firm, 2 such cover glasses were inserted back to back in one roller tube. Two to 3 hours after explantation, 2 ml of Eagle's medium were added to each tube. The cultures were incubated at 37°C, and after 3 days the cultures growing most abundantly were chosen and divided into 2 groups; experimentals and controls. The media of cultures were changed, and experimentals incubated at 37°C with serum (diluted to final concentration of 15 to 20% with Eagle's medium) from the unilaterally nephrectomized rat, obtained on 2nd and 15th postoperative days, and in one case, with serum from sham operated rat, obtained on 2nd postoperative day. Controls were incubated with serum (diluted in the same way as the experimentals) from the normal rat.

After incubation with serum for 3 days, the cultures were fixed and stained according to Jacobson's method for determination of mitotic ratios. The pancreas, anterior pituitary, and bladder of the rat (for organ specificity) and the outer medulla of puppy kidney (for species specificity) were cultured and treated in the same way. Blood was drawn aseptically from the rat by cardiac puncture using

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† Travelling Fellow of Rockefeller Fn.