shortening in the prothrombin time, equivalent to a 50% increase in prothrombin, is accomplished by an eluate without prothrombin and containing an infinitesimal amount of thrombin, suggests that the substances responsible are accessory to the main reaction and not direct contributors to the thrombin pool.

One might speculate endlessly over the synergism of SA and AHF shown in Fig. 2. We were surprised to find that supernormal prothrombic activity could not be obtained without AHF, despite the use of a potent brain thromboplastin. Possibly the simplest and least controversial inference which might be drawn from the experiments of Fig. 2 is that AHF, under certain conditions, is merely another of the numerous variables in the prothrombin time test.

Summary. 1. An eluate from $BaSO_4$-adsorption of normal serum is shown to have a marked effect on the clotting time in hemophilia without altering the defect in prothrombin utilization. 2. The eluate is shown to operate chiefly by reducing the latent period of prothrombin utilization. 3. The effect of this eluate on AHF assays is discussed. 4. The necessity of platelets for prothrombin conversion is demonstrated again under different experimental conditions. 5. AHF is shown to be required for the demonstration of maximal prothrombic activity in the prothrombin time test.

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Simplified Method for Determination of Total Adrenal Cholesterol.* (21281)

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(Introduced by E. M. Landis.)

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The determination of total adrenal cholesterol by the method of Schoenheimer and Sperry(1) or modifications thereof has been a cumbersome and time-consuming procedure. The publication of a simple and rapid direct method for the determination of serum cholesterol by Zlatkis, Zak, and Boyle(2) prompted us to adapt their procedure to the determination of adrenal cholesterol. The modified Zlatkis, Zak, and Boyle procedure was subjected to comparison with that of Sperry and Webb(3) with good agreement between the 2 methods.

Methods. Reagents. With a few minor modifications, the reagents and following procedures are those previously described by Zlatkis, Zak, and Boyle(2) for the determination of total serum cholesterol. 1. *Standard cholesterol solution*: 0.2 mg pure, dry, ash-free cholesterol/ml of glacial acetic acid (C.P. 99.7%). 2. *Ferric chloride sol.*: Dissolve 10 g

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of ferric chloride, reagent grade in 100 ml glacial acetic acid. 3. *Color reagent*: Dilute 1.0 ml of ferric chloride solution to 100 ml with C.P. concentrated sulfuric acid.

Procedure. A. Standard curve: .1, .2, .3, .4, and .5 ml of the standard cholesterol solution are pipetted into matched colorimeter tubes (19 x 150 mm) and diluted to 3.5 ml with glacial acetic acid. The blank contains 3.5 ml of glacial acetic acid. 2.5 ml of the color reagent are pipetted into each tube, allowing the color reagent to slowly flow down the side of the tube. The resulting 2 layers are then mixed rapidly by applying a brisk circular motion to the tube. This process is a critical step in the procedure and should be executed uniformly in all samples. When the tubes have reached room temperature, the absorbency is measured in a spectrophotometer at 560 mμ. B. Determination of total adrenal cholesterol: 1. Place cleaned and weighed adrenal in a McShan homogenizer(4) and homogenize the gland. 2. Add 10 ml of C.P. glacial acetic acid and mix thoroughly. 3. Filter the homogenate through Munktell's No. 00 filter paper. 4. Pipette 0.5 ml of filtrate into a 19 x 150 mm colorimeter tube. 5. Add 3.0 ml of glacial acetic acid and mix. 6. Add 2.5 ml of color reagent and mix as described above and allow to come to room temperature. 7. Read at 560 mμ as above. 8. Cholesterol content of aliquot is determined from standard curve. 9. Calculation: [Value from standard curve x 20 x 100]/[adrenal weight(mg)] = mg cholesterol/100 mg adrenal.

Experimental. In order to compare adrenal cholesterol values obtained by the direct method of Zlatkis, Zak, and Boyle with current methods based on the Liebermann-Burchard reaction, the following procedures were followed. It was first ascertained that there is no statistically significant difference between the total cholesterol concentration of the right and left adrenal glands from adult female rats of the Sprague-Dawley strain (Table I). A group of 28 normal, adult female rats of the same strain were then sacrificed by ether inhalation and the adrenal cholesterol concentration of the left adrenal was determined by the method of Zlatkis, Zak, and Boyle, while that of the right adrenal by the procedure of Sperry and Webb(3) as adapted for adrenal cholesterol by Agate(5). The results are shown in Table II, Exp. 1. It is seen that the 2 methods give identical values. To determine whether this agreement between the 2 methods would still hold following alterations in adrenal cholesterol concentration by experimental means, 3 groups of adult female rats were studied as above. One group of 6 animals served as control, a group of 14 animals was hypophysectomized and adrenal cholesterols determined by the 2 methods 7 days following operation and a third group of 10 rats was placed in a cold room at 5°C and adrenal cholesterol content was assayed following a 24-hour exposure to this temperature. The results are given in Table II, Exp. 2. The differences between the adrenal cholesterol values obtained by the 2 methods are not statistically significant. It will be noted, however, that the results of the Zlatkis, Zak, and Boyle method are somewhat higher in most instances than those of the Sperry-Webb procedure. This is in agreement with the findings of Zlatkis, Zak, and Boyle(2) for serum cholesterol. These authors attribute this slight difference to the necessity of employing several steps in methods based on the Liebermann-Burchard reaction as compared to the few steps required by their direct method with concomitant reduction in the possibility of cholesterol loss during the course of the latter analysis.

TABLE I. Total Cholesterol Concentration of Right and Left Adrenal Glands in Adult Female Rats. (Method of Zlatkis, Zak and Boyle.)

Treatment	No. of rats	Left adrenal	Right adrenal	P
None	12	5.40 ± .24*	5.56 ± .26	>.1

* Mean ± stand. error of mean.

TABLE II. Cholesterol Concentrations in Left Adrenal (Zlatkis, Zak and Boyle) vs. That in Right Adrenal (Sperry-Webb) in Normal, Hypophysectomized and Cold Stressed Rats.

Exp.	Treatment	No. of rats	Left adrenal (ZZB)	Right adrenal (SW)	P
I	None	28	5.10 ± .17*	5.12 ± .22	>.1
II	"	6	4.78 ± .35	4.17 ± .38	"
	Hypox.	14	10.68 ± .31	10.12 ± .35	"
	Cold	10	2.44 ± .18	2.18 ± .16	"

* Mean ± stand. error of mean.

Conclusions. The method of Zlatkis, Zak, and Boyle for the direct determination of serum cholesterol has been adapted to the determination of total adrenal cholesterol. The values thus obtained do not differ significantly from those obtained by the method of Sperry and Webb in normal, hypophysectomized and cold stressed animals. The time required to analyze 10 samples and 5 standards by the Zlatkis, Zak, and Boyle procedure requires less than one hour compared to the 2 days usually necessary when methods based on the Liebermann-Burchard reaction are utilized.

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Hyaluronic Acid Formation by *Streptococcus pyogenes.** (21282)

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Previous work(1) with 4 encapsulated strains of *S. pyogenes* demonstrated that these microorganisms form from 30 to 50 mg of hyaluronic acid per 100 ml of culture when grown for 12 hours in 1% glucose infusion broth. When galactose is substituted for glucose as the carbon source only 5 to 10 mg of the capsular polysaccharide can be detected per 100 ml of culture in the same period of time. That the substance found in such cultures is hyaluronic acid is indicated by our observation that it is susceptible to hydrolysis by both streptococcal and testicular hyaluronidases and that it precipitates under the same conditions as does the material from the glucose-containing cultures.

It has been reported(2,3) that the intact carbon skeleton of glucose is incorporated into the structure of hyaluronic acid by *S. pyogenes* in glucose-containing media. No data are available on the mechanism of capsule synthesis in the presence of galactose but certain factors which influence the production of hyaluronic acid in both glucose and galactose broth cultures of *S. pyogenes,* strain S23, will be reported here.

Methods. Organism S. pyogenes, strain S23, a type 14 mucoid organism, was used throughout. *Culture Media* Two and one-half per cent heart infusion broth (Difco) supplemented with 0.5% neopeptone (Difco) and 1.0% sugar was employed. The medium was adjusted to pH 7.5, diluted to 98% of final volume and autoclaved. Sufficient sterile 10% $NaHCO_3$ was then added to bring the medium to volume. The final pH, before inoculation, was approximately 7.8. *Cultivation* Culture stocks were maintained on coagulated whole egg slants with transfers at monthly intervals. Blood agar plates were streaked periodically to detect dissociation. This strain displayed a uniform capacity to synthesize hyaluronic acid when maintained under these conditions. Inocula, consisting of the centrifuged cells from 17 hour cultures in the standard medium modified to contain 0.2% glucose, were used in the hyaluronic acid formation studies. The volume of each inoculum culture was one-tenth that of the medium to be inoculated. Incubation of all cultures was at 37° C. *Quantitative Determinations* For the estimation of bacterial nitrogen and hyaluronic acid, two 5 ml aliquots of each culture were removed and centrifuged. The supernates were carefully withdrawn, pooled, and the individual cell sediments washed once with 5 ml of saline. Nitrogen determinations were performed as previously reported(1). The pooled supernates were heated in a water bath at 60° C for 45 minutes to remove trace amounts of

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