of relationship between the spermatozoa of different species.

The basic principle of the test, however, would seem to have a general application to any agglutinative technic involving large motile organisms, where the agglutinates formed are so fragile as to be readily disrupted by shaking or jarring. It may perhaps be of value in the study of trypanosomes and other large flagellated protozoa. Although gelatin has proved suitable for the enhancement of sperm agglutination by increasing the viscosity of the medium, different agents may be better suited for other motile organisms.

Summary. 1. An improved macroscopical agglutination test for the detection of agglutinins against human spermatozoa is described. 2. The test employs an antigen of actively motile spermatozoa, and a final concentration of 2.5% gelatin to increase the viscosity of the medium. 3. The viscous gelatin medium prevents dissolution of the weakly bound sperm agglutinates in the higher antiserum dilutions, and retards sedimentation so as to render resuspension unnecessary. 4. The new method appears to be highly sensitive and specific. Agglutinative titers ranging from 1:16,000 to 1:256,000 with a mean of about 1:60,000 have been obtained with 7 anti-human-sperm rabbits' sera. These titers are over 100-fold higher than those obtained with the usual agglutinative technics. 5. The possible applications of the test are suggested.

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Relation between Adrenal and Pituitary Glands and Urinary Excretion of Neutral Reducing Lipids in the Rat.* (19741)

H. SOBEL, J. MARMORSTON, H. GREENFELD, H. C. GOODMAN,† A. L. SELLERS, AND S. SMITH III.‡

From the Department of Biochemistry, Division of Laboratories and the Institute for Medical Research, Cedars of Lebanon Hospital; and the Department of Medicine, University of Southern California School of Medicine, Los Angeles.

Adrenal cortical stimulation in the rat is, at present, evaluated by adrenal ascorbic acid or cholesterol depletion in acute experiments (1) and by adrenal weight and morphological changes in chronic experiments(2). During the course of studies on the role of the adrenal in experimental hypertension, it was thought desirable to evaluate adrenal cortical activity by measuring the urinary excretion of adrenal cortical hormones or their metabolites. Several chemical procedures(3-5) have been developed for the determination of these substances in human urine. The method of Heard and Sobel(3) is based on the ability of neutral lipid extracts of urine to reduce phosphomolybdic acid. This method has been shown to correlate with clinical and biological evidence of altered adrenal cortical activity in human beings. In the present study, a modification of this method has been applied to evaluate adrenal cortical activity in the rat.

Experimental procedure. Urine collection. The method for urine collection has been described(6). Rats of the Slonaker-Addis strain were taken off the stock diet and placed in urine collection cages at 5 P.M. During the overnight collection period the animals received *ad libitum* a 15% glucose solution in 0.4% sodium chloride containing 0.5% of a mixture of B vitamins (Betaplexin®). There may be some theoretical objections to the use of this solution in experiments designed to test adrenal cortical function. Nevertheless, it was necessary in order to assure a copious flow of uncontaminated urine. Normal rats on over-

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† Howard R. Hughes Medical Research Fellow.

‡ Columbia Foundation Fellow in Medical Research.

night collection drink approximately 50 to 60 ml of this mixture and excrete approximately 40 to 50 ml of sugar-free urine. Since the neutral reducing lipid material is rapidly destroyed in alkaline solution, 2 g of ammonium sulfate were added to the urine bottles prior to the collection period. The following morning the urines of each experimental group were pooled and filtered through glass wool. The pH of the pooled specimens was usually between 6.0 and 6.5. If the pH exceeded 7.0 the urine was discarded.

Chemical procedure. A 15 ml aliquot of urine was adjusted to pH 1.0 with 6 N hydrochloric acid with the aid of a Beckman pH meter. The urine was transferred to a 30 ml ground glass stoppered test tube and shaken with 4 ml of a 4:1 ether-chloroform mixture. The supernatant was aspirated into a separatory funnel. This operation was repeated 3 times. The combined extracts were shaken 4 times with 3 ml portions of ice cold 0.1 N sodium hydroxide and 4 times with 3 ml portions of water; then dried over anhydrous sodium sulfate. The extract was transferred to a colorimeter tube and the solvent was removed under a stream of nitrogen. Five ml of a 1:1 mixture of glacial acetic acid and phosphomolybdic acid solution (Folin-Wu) were added and the contents of the tube were heated for 60 minutes in a boiling water bath. A standard solution containing 100 γ of desoxycorticosterone was similarly treated. The color was read at 660 μ. The results were calculated as γ equivalents of desoxycorticosterone per rat per day. The calculated value is the amount of neutral reducing lipids (NRL) contained in the urine per rat per day.

Biological procedure. Bilateral adrenalectomy was performed in one stage under ether anesthesia. The adrenalectomized rats were maintained on a 1% sodium chloride solution for 4 to 5 days after the operation.

Beef pituitaries were carefully dissected and the anterior and posterior lobes were enucleated. The preparations were made by brief maceration of these fractions with acetone in a Waring Blendor. The residue was filtered, dried, then ground to a fine powder. These pituitary suspensions, as well as ACTH and growth hormone, were administered in 0.9% sodium chloride solution subcutaneously in the dosages indicated in the text. Control animals received 0.9% sodium chloride solution only.

TABLE I. Effect of Adrenalectomy and Administration of Pituitary Hormones on Neutral Reducing Lipid Excretion in the Rat.

Group	No. of rats	No. of observations	NRL (mean	stand. dev.)	"p"*
Normals	600	150	61.2	2.1	
Adrenalectomy	104	26	29	1.9	.001
ACTH	74	14	74	8.1	.1
Growth hormone	27	7	91	13.9	.03
Ant. pituitary	24	6	78	6.8	.2
Post. "	24	7	191	17	.001

* "p" compares each mean to the mean of the normal controls.

Results. The data were analyzed by Fisher's Method. "p" values greater than 0.01 were not considered to be significant. The NRL excretion usually ranged between 50 and 90 γ per rat per day (mean 61.2 $\pm$ 2.1, Table I). However, since these limits were in some instances exceeded, it was necessary to compare the excretions of the experimental animals with that of their controls in any individual determination. There was no significant sex difference in the level of NRL excretion; male rats excreted 65 $\pm$ 2.7, while female rats excreted 57 $\pm$ 3.1 γ per rat per day. The difference between these means is not significant.

Adrenalectomy. The excretion of NRL by adrenalectomized rats was determined on 104 rats in 26 groups carried out over a period of nearly 2 years. The average daily excretion was approximately 29 γ per rat.

ACTH administration. It has been observed in this laboratory that rats of the Slonaker-Addis strain do not have an eosinopenia following a single injection of ACTH§ (7). It was necessary to give this substance in 3 doses in order to obtain a maximal eosinophile fall. ACTH was, therefore, given at 5 P.M., 11 P.M., and 7 A.M., during the overnight collection period. The total dosage

§ We wish to thank Doctors Edwin E. Hays and Irby Bunding of Armour and Company, Chicago, Illinois for supplying us with the ACTH and the growth hormone used in this experiment.

administered per rat ranged from 8 to 48 mg equivalents of LAIA standard. This was given to 74 rats in 14 groups. In only 3 instances was an average increase in NRL excretion obtained. It is concluded that ACTH does not significantly increase NRL excretion in the strain of rats used in this study.

Growth hormone administration. It has been suggested that growth hormone is involved in stimulating the production of mineralocorticoids by the adrenal cortex(8). Growth hormone§ was administered subcutaneously in doses of 0.5 mg to 12.5 mg per rat at the start of the urine collection period. In 6 out of 7 experiments, no significant change in excretion was noted. It is concluded from these data that growth hormone does not increase NRL excretion.

Administration of acetone dried anterior and posterior pituitary powder. Several pilot experiments indicated that saline suspensions of beef whole pituitary glands produced large increases in NRL excretion, whereas similarly treated suspensions of casein or rat liver powder in 0.9% sodium chloride solution were inert in this respect. Anterior and posterior pituitary gland preparations were then tested.

Fifty mg of preparation was suspended in 4 ml of 0.9% sodium chloride solution and injected subcutaneously at the beginning of the urine collection period. An equivalent amount of 0.9% sodium chloride solution was injected into the control rats.

Anterior pituitary powder produced no significant change in NRL excretion. Except in one instance, posterior pituitary powder produced excretions exceeding 160 γ per rat per day. The greatest quantity excreted by a group of normal rats was 124 γ per day. Following the administration of posterior pituitary preparation, the rats become lethargic for a period of several hours. This effect has been noted in dogs and rabbits and is described as a state of "apnea"(9). The output of urine was greatly decreased. In a single instance when NRL excretion did not increase following posterior pituitary injection, the oliguria and lethargy were still present. These symptoms were present after the administration of heated posterior pituitary suspension, although the preparation had lost, to a considerable degree, its ability to increase NRL excretion.

TABLE II. Effect of Posterior Pituitary Powder on Neutral Reducing Lipid Excretion in Adrenalectomized Rats.

Treatment*	No. of rats	Urine vol, ml/rat/17 hr	NRL, γ/rat/day
Normal	13	44	66
Normal post. pituitary powder	13	9	184
Adrenalectomy	18	34	36
Adrenalectomy post. pituitary powder	18	8	73

* All animals received 4 cc of .85% sodium chloride solution subcutaneously in addition to the indicated treatment.

Several preliminary experiments seem to indicate that pitocin‖ is inert, but that pitressin¶ does increase NRL excretion. Thus, totals of 7.5, 15, and 30 units of pitressin, given in 3 injections, caused an excretion of 95 γ, 144 γ, and 153 γ per rat per day. The control rats excreted 75 γ per rat per day.

Administration of posterior pituitary powder to adrenalectomized rats. It is possible that posterior pituitary powder increases NRL excretion by stimulating the adrenal cortex. In order to test this hypothesis, posterior pituitary powder was administered to adrenalectomized rats and the results are indicated in Table II. Posterior pituitary powder caused an average increase in excretion of 118 γ above normal while in adrenalectomized animals an average increase of 37 γ above the saline injected adrenalectomized controls was produced.

Discussion. The use of phosphomolybdic acid as a reagent for the determination of NRL is based upon the ability of the —CO CH_2OH group and the $\alpha:\beta$ unsaturated —3-ketone of ring A of the steroid nucleus to reduce the reagent(10). When applied to human urine, there is a correlation between the level of NRL excretion and the biological activity of equivalent extracts, although a fraction of the NRL is not of adrenal cortical

‖ Parke Davis brand used, 10 pressor units per 1 cc vial.

¶ Parke Davis brand used, 20 pressor units per 1 cc vial.

origin(3). The NRL determination in human urine has been used clinically to evaluate adrenal cortical function(11,12). The administration of ACTH to man causes a considerable increase in the excretion of neutral reducing lipids(13). Burstein(14) has reported that a large increase in excretion of NRL and formaldehydogenic material followed the administration of as little as 100 γ of ACTH to the guinea pig. It was, therefore, startling to observe that even though adrenalectomy reduces NRL excretion to half the control value, doses of ACTH which produce an eosinopenia in the Slonaker-Addis strain of rats do not increase NRL excretion. The failure of anterior pituitary powder to increase NRL excretion confirms the lack of effectiveness of ACTH.

It was even more surprising to observe that an increased NRL excretion was induced by posterior pituitary gland powder. The causative agent in this crude mixture appears to be thermolabile. The substance which increased NRL excretion probably acts through the adrenals, although some increase in excretion does occur in the absence of the adrenals.

Summary. The neutral reducing lipids were determined in the urine of rats. Adrenalectomy decreased the excretion of this material to one-half the control value. Neither ACTH, growth hormone, nor anterior pituitary powder significantly increased NRL excretion. Posterior pituitary powder increased the excretion approximately 3-fold. This response is considerably diminished in adrenalectomized rats.

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Influence of Influenza Hemagglutinin on Hemolysis Rates. (19742)

J. B. BATEMAN, MARY S. DAVIS, AND PATRICIA A. MCCAFFREY.
From Camp Detrick, Frederick, Md.

Much of what is known about the red cell membrane has resulted from observing the effects of various chemical substances upon red cells. Certain of these when added to a cell suspension become attached to the cell surface. This attachment or its sequelae may manifest themselves in many ways, agglutination or lysis being among those most readily observed and least understood.

Although hemagglutinating and hemolysing substances are not always sharply differentiated, there are instances in which the two types of action can be separated quite clearly. In this report, which deals with the successive actions of the two types of substance, the guiding principle has been the idea that effects might be produced which could be interpreted as the result either of simple blockage of one function by the other or of complete independence of the two functions,