However, a second explanation was suggested by the report[11] that antifibrinolysins differed immunologically. If antibody differences were a significant factor in the determination of antifibrinolysin, it seemed possible that the detection of antibody rises might be limited to the variety of fibrinolysin employed as antigen. The present study was consequently undertaken to test for and, if possible, to determine the nature of the differences in antifibrinolysins.

An attempt was made to obtain evidence of such differences by measuring the antibody level of the sera of patients infected by streptococci belonging to different Lancefield groups. Quantitative antibody titrations were carried out on the sera of patients with infections due to organisms from each of the 3 groups, A, C and G; and the fibrinolysins used for testing for antibody were derived from strains from each of these same groups. The results of the study showed that the titers of the various sera tested were essentially the same with all 3 fibrinolysins.

It would thus appear that the fibrinolysins produced by these different organisms were immunologically identical in their reactivity. This is in agreement with the observations of other workers.[3,10,26] It is not possible to explain the different results reported in the first quantitative studies of the problem.[11] However, as possibly contributing to apparent differences, 2 points may be mentioned: (1) the nonspecific factors in the plasma, such as antiprotease or a deficient lytic factor, may have produced the differences observed, or (2) the variations may have been due to the method of antibody estimation employed. Measurement of antibody was based on the amount of fibrinolysin which permitted clot dissolution. Since fibrinolysin and antifibrinolysin combine in varying multiple proportions,[15] it would appear that a procedure in which the fibrinolysin concentration is varied is not suitable for the measurement of antibody. The evidence thus suggests that the fibrinolysins produced by streptococci pathogenic for man are identical in their immunological behavior.

Conclusion. It was concluded, therefore, that the use of a single fibrinolysin preparation is satisfactory for the serological determination of antifibrinolysin in human infections.

[26] Kirby, W. M., and Rantz, L. A., *Arch. Int. Med.*, 1943, **71**, 620.

15490

Action of Estrogen on Release of Hypophyseal Luteinizing Hormone.

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The gonad stimulating properties of the pituitary are better understood than is the reciprocal action of the gonadal secretions on the pituitary. In respect to the latter process the terms "suppression" and "inhibition" are frequently used to describe what is obviously a more complicated mechanism than these empirical terms imply. The pituitary gonadotropic complex does not always act as a unit and individual factors should be considered separately in their response to estrogens and androgens. Furthermore, the intracellular synthesis of a pituitary factor is a process distinct from that involving the liberation of the factor from the gland.

We have attempted in the work presented here and in our previous studies,[1,2] to obtain

[1] Hellbaum, A. A., and Greep, R. O., *Am. J. Anat.*, 1940, **67**, 287.

[2] Hellbaum, A. A., and Greep, R. O., *Endocrinology*, 1943, **32**, 33.

TABLE I.
Comparison of the Residual Pituitary Gonadotropic Activity Found in: Normal Adult Female Rats; Female Rats 3 Months After Spaying; Spayed Female Rats Treated with Estrogen for 30 to 45 Days Beginning at 3 Months After Spaying.

Adult female donors			Immature recipient female rats			
				Ovarian wt		
Type	No. of animals	Avg dose (mg)	No. of animals	Avg (mg)	Range (mg)	Type of ovarian response
Normal 21-day-old female recipients.						
Intact	38	14.5	7	29.1	16- 49	2 to 5 corpora lutea
Spayed 3 months	8	3.6	8	73.9	32-135	Numerous corpora lutea
	6	1.7	12	39.0	23- 54	" " "
Spayed 3 months then treated with 15μ estradiol benzoate 30 to 45 days	14	13.8	7	31.3	20- 42	6 rats with follicles only 1 rat with corpora lutea
Avg ovarian wet of uninj. immature control rats—12 mg.						
Hypophysectomized Female Recipients						
Intact	40	19.2	5	27.2	21- 34	2 to 5 corpora lutea
Spayed 3 mo	9	3.54	9	54.8	34-103	Numerous corpora lutea
	5	1.7	10	32.4	19- 56	" " "
Spayed 3 mo then treated with 15μ estradiol benzoate 30 days	7	6.4	7	20.2	16- 28	Follicles only
Spayed 3 mo then treated with 3μ estradiol benzoate 30-45 days	9	5.8	9	15.4	9- 26	" "
Avg ovarian wgt of 8 hypophysectomized immature controls—8.7 mg.						

information on the storage and release of pituitary gonadotropins. The purpose of this report is to demonstrate the qualitative changes in the residual luteinizing component of the pituitary at various levels of estrogen influence.

The experimental procedure consisted of comparing the type of ovarian stimulation produced by the pituitaries of: (1) normal adult female rats; (2) adult female rats 3 months after the removal of the ovaries; (3) oophorectomized adult female rats injected with estradiol benzoate after a 3-month postoperative interval. The pituitaries of the different donor groups were assayed in normal female rats 21 days of age and in immature hypophysectomized female recipients.

The donor pituitaries, following removal, were dehydrated in acetone, dried, and powdered by the procedure previously reported.[1] The powder was suspended in water and injected twice daily for 3 days into normal and hypophysectomized recipients. The latter received their first injection 48 hours after the operation. Necropsies were made 120 hours after the first injection. The ovaries were weighed and examined before fixation for follicle growth and for presence of corpora lutea. Histological sections were prepared when corpora lutea were not grossly visible. The data are summarized in Table I.

Normal Adult Female Rats. Assays carried out on the pituitaries of this group revealed the characteristic gonad stimulating activity of pituitaries which have been subjected to the normal output of ovarian hormones. A total of 78 glands were assayed in 7 normal and 5 hypophysectomized recipients (Table I). The resultant ovarian responses showed that the pituitary gland

of the normal rat contains moderate amounts of the luteinizing hormone. There were from 2 to 5 corpora lutea in the ovaries of each of the recipients.

Spayed Adult Female Rats. In this group, 28 adult female rats were oophorectomized 3 months prior to necropsy and removal of the pituitary glands for assay. A total of 20 normal and 19 hypophysectomized recipients were used. The pituitaries from this donor group showed a marked increment in the luteinizing factor. The ovaries of the recipient rats were extensively luteinized. Macroscopic and histologic examination revealed ovaries filled with corpora lutea and few, or no maturing follicles.

In addition to an increase in the residual luteinizing factor, following spaying, the pituitary content of follicle-stimulating hormone was likewise increased. Less than 1/10 of the dose of pituitary powder from spayed rats was required to produce an ovarian weight increase comparable to that produced by the pituitaries of normal animals. This was undoubtedly due, in part, to an increased storage of the luteinizing hormone which acted synergistically with the follicle-stimulating hormone to produce greater ovarian enlargement.

Oophorectomized Adult Female Rats Injected with Estradiol Benzoate. The object of this experiment was to determine whether the store of luteinizing hormone, which had accumulated in the pituitary as a result of spaying, could be released by estrogen injection and be reduced to such a degree that its physiological activity would be undetectable in recipient female rats.

A total of 21 adult animals, which had been spayed 3 months previously, were injected daily with 15 μ estradiol benzoate* in oil for 30 to 45 days. The pituitaries of these animals were tested in 7 intact and 7 hypophysectomized recipients. The ovaries of only one of the assay animals contained corpora lutea. The ovaries of the remaining 13 animals showed only follicle stimulation. The presence of lutein tissue in the single intact recipient may have been due to endogenous luteinizing hormone from the test animal's own pituitary gland.

Another group of 9 adult oophorectomized rats were injected with 3 μ of estradiol benzoate daily for periods varying from 30 to 45 days. These pituitaries were administered to 9 hypophysectomized recipients. The ovaries of none of these animals showed a trace of luteinization, although most of them had well developed follicles.

It is clear from these data that estrogen caused a liberation of the luteinizing hormone to a point where this substance was being released as rapidly as it was being produced. This finding is in accord with our previously expressed views regarding the effect of the gonads on the pituitary. Although our former study[2] was concerned primarily with the action of testosterone, we doubt that there is an essential qualitative difference between the action of estrogen and testosterone on the pituitary with respect to their ability to cause a release of the luteinizing hormone. This view concurs with the findings of other workers investigating functional activity of the pituitary, with respect to gonad stimulation, during treatment with sex hormones.[3-7] Laqueur and Fluhmann[8] injected testosterone into normal female rats and obtained a diminution of gonadotropic potency (by implants) which they attributed to an inhibition of the production of luteinizing hormone.

Summary. The pituitaries of adult female rats, under normal estrogen influence, stimulated moderate luteinization in normal and hypophysectomized recipient immature female rats. The pituitaries of adult female rats freed of estrogen stimulation through oophorectomy, invariably caused extensive luteinization of the recipient ovaries. This

* The estradiol benzoate was kindly furnished by the Schering Corporation, Bloomfield, N.J.

3 Hohlweg, W., *Klin. Wchnschr.*, 1934, **13**, 92.

4 Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1377.

5 Wolfe, J. M., and Hamilton, J. B., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 189.

6 Fevold, H. L., Hisaw, F. L., and Greep, R. O., *Am. J. Physiol.*, 1935, **114**, 508.

7 Freed, S. C., Greenhill, J. P., and Soskins, S., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 440.

8 Laqueur, G. L., and Fluhmann, C. F., *Endocrinology*, 1942, **31**, 300.

indicated that liberation of the hypophyseal luteinizing factor was minimal in the absence of estrogen; the factor remaining stored within the pituitary gland.

Release of the luteinizing factor by estrogen was suggested also by the type of ovarian response produced by pituitaries from oophorectomized adult female rats which had been injected with estradiol benzoate daily for 30 to 45 days. These pituitaries produced follicular development but no corpora lutea, due to removal of the luteinizing factor by the estrogen treatment.

15491 P

Effect of Long Chain Fatty Acids on Bacterial Growth.

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We have shown elsewhere that certain water soluble lipids promote diffuse growth of tubercle bacilli in synthetic media, especially in the presence of serum albumin.[1,2] These same substances have now been found to enhance the growth of other microbial species, in particular of an unidentified micrococcus (strain C), recently isolated in our laboratory. Some understanding has also been gained of the conditions under which long chain fatty acids can stimulate bacterial growth.

It is known that the soaps of fatty acids exert a bacteriostatic and bactericidal effect on certain micro-organisms, particularly on the Gram-positive and acid-fast species, and that unsaturated acids are more toxic than the corresponding saturated compounds.[3-9] For example, concentrations of oleic acid as low as 0.000001-0.00001% are sufficient to cause inhibition or retardation of growth of small inocula of human tubercle bacilli in synthetic liquid media. On the other hand, fatty acid esters (methyl oleate, triethanolamine oleate, phosphatides) exhibit little or no primary toxicity. That the lack of toxicity is not due to poor solubility of the esters is indicated by the fact that the polyoxyethylene derivatives of oleic acid are essentially nontoxic, even though they are completely dispersible in water.[1,2] Thus, tubercle bacilli grow readily in synthetic media to which has been added 0.1-1.0% of Tween 80 (a polyoxyethylene derivative of sorbitan monooleate) purified to remove unesterified fatty acid.[10] Detoxification of