lines, and make up 39% of all the animals injected with testosterone. This reaction was observed more frequently during February and March than at any other time, the entire period of the experiments lasting from May, 1944, to April, 1945. The hormone preparation was the same before and after that period, so that variations in the testosterone can be excluded as the cause. Perhaps of still greater interest are the observations that these animals did actually show some degree of hypertrophy suggesting some form of response, and that they showed earlier the changes in composition which are typical for regression, such as loss in water and rise in protein and lipids. They formed a distinct group. No explanation can be offered as to the mechanism responsible for the lack of maximal response.

Summary. The changes in weight and in concentrations of water, protein, lipids, and ascorbic acid of the corpus luteum of the rat during pregnancy and during periods of injections with testosterone propionate at two dosage levels are reported. Comparison of the results permits the conclusion that the changes in weight and in chemical composition of the corpus luteum in experimentally induced hypertrophy are essentially the same as those occurring in untreated rats during the course of pregnancy.

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Comparison of the Cephalin-Cholesterol Flocculation with the Thymol Turbidity Test.

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The thymol turbidity test has been described recently by Maclagan[1] as an index of parenchymal liver dysfunction. Because of its simplicity the test gives promise of being of considerable aid in detecting disorders of the liver in which changes in the serum proteins are present. In clinical practice there is a marked parallelism between the thymol test and the cephalin flocculation test which also depends upon alterations of the serum pattern in certain liver diseases. Our studies indicate, despite this agreement, that the mechanisms of the two tests differ in important respects. In this paper some of these differences are described.

The thymol test is performed by adding 0.05 cc of the serum to be tested to 3 cc of saturated thymol in barbiturate buffered distilled water (pH 7.8). The intensity of the turbidity which develops within 10 minutes indicates the degree of active liver damage. Buffered distilled water without the addition of thymol does not give a positive test.

We have confirmed the findings of Maclagan that the test is usually positive in cirrhosis, hepatitis, etc., in which the cephalin flocculation is also positive. Notable exceptions have been observed, *e.g.*, the thymol test was positive and the flocculation negative in (1) certain lipemic sera such as in diabetes and in nephrosis, (2) biliary cirrhosis, (3) certain cases of metastatic neoplasm of the liver, (4) convalescent hepatitis, and (5) certain normal sera. The thymol test was negative and the flocculation positive in normal laboratory animals such as dogs and rabbits, and in certain rare cases of presumably normal individuals with no demonstrable liver disease.

Fundamental differences in the mechanism of the two tests can be demonstrated by studies on (1) effect of NaCl, (2) removal of lipids by

[1] Maclagan, N. F., *Nature*, 1944, **154**, 670; *Brit. J. Exp. Path.*, 1944, **25**; Hanger, F. M., *Tr. Assn. Am. Phys.*, 1938, **53**, 148; *J. Clin. Invest.*, 1939, **18**, 261.

ether extraction of frozen serum, (3) electrophoretically separated serum fractions, and (4) serum separated by ultracentrifugation.

1. *Effect of NaCl.* If the standard thymol solution is prepared with physiological salt solution instead of distilled water, all tests become negative. In contrast, the cephalin flocculation is routinely performed in salt solution which has no effect on the development of flocculation or precipitation.

The presence of salt in serum is probably accountable for the prozone noted in the thymol turbidity test. When a series of tubes containing reactive serum and a standard solution of thymol in distilled water were prepared (1/1, 1/2, 1/4, 1/16 . . . .) maximum turbidity was noted at 1/16. After removing the electrolytes from the serum by dialyzing against distilled water, turbidity was noted in the lower dilutions also.

2. *Effect of extraction of lipids from serum.* Sera from cases of hepatitis, malaria, and other disorders giving a positive thymol test were extracted by freezing in the presence of ether, as described by McFarlane,[2] a technic that has been shown to remove a large proportion of the serum lipids. In most instances the thymol test was made negative or weakly positive by ether extraction, while the cephalin flocculation test remained unaffected. Freezing alone had no effect on the reactions of the sera. Some exceptions were encountered in an occasional case of hepatitis, malaria, and disseminated lupus erythematosus where the thymol test remained positive after ether extraction.

The addition of whole normal serum, in equal amounts, to whole hepatitis serum, had no effect on the intensity of the thymol test, while the intensity of the cephalin flocculation reaction was strikingly reduced. On the other hand, the addition of whole normal serum to extracted hepatitis serum restored a negative thymol test to its original intensity. Extracted normal serum added to extracted hepatitis serum had no effect, and when extracted normal serum was added to unextracted hepatitis serum, the intensity of the thymol test was markedly reduced. Extracted normal serum could not be made positive by the addition of whole normal serum.

These findings tend to confirm the implication of Maclagan that lipids are essential to the thymol reaction. Further proof of this assumption was supplied by the finding that the addition of aqueous suspensions of cephalin, crude lecithin, or cholesterol, to extracted hepatitis sera restored a positive thymol reaction. Aqueous suspensions of the residues of the ether extracts from normal or hepatitis sera exhibited similar reactivating powers. On the other hand, there is no evidence that lipids play an essential part in a positive cephalin flocculation reaction, since there is no demonstrable effect on the intensity of the reaction after extracting or adding the lipids mentioned above.

3. *The thymol test on electrophoretically separated serum fractions.* Albumin and gamma globulin fractions from normal and abnormal sera were obtained by electrophoretic separation in phosphate buffer of pH 7.4. Negative thymol tests were observed with all albumin and gamma globulin fractions from both normal and abnormal sera. The addition of lipids and ether extract residues of serum to these fractions failed to produce a positive thymol test.

In contrast, gamma globulin fractions from normal and from hepatitis sera, as has been shown previously,[3] gave positive cephalin flocculation reactions. Albumin fractions from normal serum inhibited the flocculation of normal and hepatitis gamma globulins, while the albumin fraction from hepatitis serum failed to inhibit flocculation.

4. *The thymol test on serum fractions obtained by ultracentrifugation.* Whole hepatitis sera, extracted hepatitis sera, and extracted normal sera were centrifuged at 48,000 RPM for 3 hours. The sera were separated into three layers: (1) a clear supernatant, (2) a bilirubin stained middle layer, and (3) a small pellet. Flocculations and thymol test were set up with each layer. The supernatant layer from all 3 sera gave negative thymol and flocculation tests. The pellet gave positive flocculation and negative thymol reactions in all sera. The middle layer of whole hepatitis

[2] McFarlane, A. S., *Nature*, 1942, **149**, 439.

[3] Hanger, F. M., Moore, D. B., Pierson, P. S., and Moore, D. H., *J. Clin. Invest.*, 1945, **24**, 3, 292.

serum gave a positive thymol test, while the middle layer of normal serum and of extracted hepatitis serum gave negative thymol tests. The flocculation test was positive with the middle layer in all sera. The serum component giving a positive flocculation reaction appears to be present in both the middle layer and the pellet while that responsible for a positive thymol test is only in the middle layer, where the presence of lipid is also a requisite.

Discussion. Notable differences in the mechanisms of the cephalin flocculation and the thymol test are shown in the observations recorded above. The gamma globulin fraction, upon which the cephalin flocculation depends, is not an essential factor in the thymol test. Also the electrophoretically derived albumin fraction has no effect on the intensity of the thymol test. On the other hand the albumin fraction is an important factor in the flocculation reaction, since normal albumin inhibits flocculation while that derived from hepatitis serum fails to inhibit flocculation.

The presence of lipids is necessary for a positive thymol test, but has little effect on the cephalin flocculation reaction. There is no evidence that an abnormal lipid is present in hepatitis serum since the negative thymol test obtained after ether extraction can be made positive once more by a variety of lipid preparations and even by whole normal serum. Lipids alone give no reaction with the thymol reagent.

It must be assumed that an abnormal constituent in the serum in addition to lipids is required for a positive thymol test in cases of liver diseases. This assumption is based upon the observation that the addition of normal serum or of various lipid preparations restores a positive reaction only to extracted serum which was thymol positive before extraction. The disturbance lies apparently in the serum globulin fraction, but, as has been shown, does not involve primarily the gamma globulins.

Conclusions. The thymol turbidity test tends to parallel the cephalin flocculation test in most liver disorders and other disease, but notable discrepancies are frequent. Fundamental differences in the mechanisms of the two tests have been pointed out. Notable among these are, (1) the effect of salt on the 2 reactions, (2) the importance of lipids, (3) the role of electrophoretically derived albumin fractions and gamma globulins, and (4) the comparative reactions of serum fractions obtained by ultracentrifugation. It is recommended that both tests be employed for clinical purposes since different disturbances in the serum complex are detected by each.

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Blood Sugar Level and Autonomic Balance.*

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Previous investigations of Gellhorn and collaborators[1] have shown that the excitability of the sympathetic vasomotor center is increased with falling blood sugar level as indicated by the increased blood pressure response of man and animals to anoxia and to hyper-

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[1] Gellhorn, E., Ingraham, R. C., and Moldavsky, L., *J. Neurophysiol.*, 1938, **1**, 301; Ingraham, R. C., and Gellhorn, E., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 315; Kraines, S. H., and Gellhorn, E.,