produces consistently a slightly greater acid production than does thymine, it appears that the imino compounds act as such and not, as might have been supposed, primarily by deaminization to thymine. The action of the thio compounds is somewhat more complex. Thus, 2-thio- and 2,4-dithiothymine have only slight activity, whereas 4-thiothymine is approximately as active as thymine, perhaps due to the lability of the 4-thio atom.

The introduction of a substituent into the 6-position of the thymine molecule seems, in general, nearly to abolish the activity. Thus a second methyl group as in 2,4-dioxy-5,6-dimethyl or an amino group in the same position renders the compound essentially inactive.

As shown below, if the methyl group of thymine is replaced by an amino or hydroxy group, compounds with a considerable inhibitory activity are produced. The reverse change, replacement of the oxy group in the 2-position by a methyl group, might have been expected to produce a compound with similar properties. However, the compound formed, 2,5-dimethyl-4-oxypyrimidine, is essentially inactive. This is, perhaps, related to the fact that the oxygen atoms of thymine apparently exist in the doubly bonded lactam form.[7] The entrance of a methyl group in the 2-position, therefore, involves a considerable change in the configuration of the molecule, since it results in the elimination of one imide hydrogen atom.

Among the 5-substituted uracils, compounds which may be regarded as derived by the substitution of other groups for the methyl group of thymine, there were found a number of compounds which inhibit the growth of *L. casei*. Isobarbituric acid (5-hydroxyuracil) and 5-aminouracil have approximately the same molecular weight and would be expected to have a spatial configuration similar to that of thymine. Both strongly inhibit the growth of *L. casei*. Moreover, growth can be restored by increasing the concentration of either thymine or folic acid. It is reasonable to suppose, therefore, that these substances exert their action by the displacement of thymine from active enzyme centers or structural elements and suggest the possibility of a metabolic function of the methyl group which can not be performed by the hydroxy or amino groups. Since the effects of these two inhibitors are exerted with either nutrilite, these observations are not inconsistent with Stokes's view of thymine as a product of an enzyme system which involves folic acid as a prosthetic group or coenzyme.[1] That this hypothesis is not an adequate explanation of the action of thymine, however, is brought out by a consideration of the effects of the 5-halogenopyrimidines and of 5-nitrouracil. For example, bromouracil can inhibit completely the growth of *L. casei* with thymine as the nutrient but may have no effect or produce a slight stimulation when folic acid is used as the nutrient. It follows that the growth of *L. casei* with folic acid as nutrient does not depend on an intermediary synthesis of thymine as such. Moreover, nitrouracil can prevent the growth of *L. casei* with folic acid at concentrations which have little or no effect on the growth with thymine. These observations suggest that the two nutrients act, in some fashion, as alternatives rather than as two components of an anabolic system.

GEORGE H. HITCHINGS
ELVIRA A. FALCO
MARION B. SHERWOOD
THE WELLCOME RESEARCH LABORATORIES,
TUCKAHOE, N. Y.

[7] J. R. Loofbourow, Sr. Miriam Michael Stimson, O.P., and Sr. Mary Jane Hart, O.P., *Jour. Am. Chem. Soc.*, 65: 148, 1943.

THE THYMUS AND ACETYLCHOLINE SYNTHESIS

THE possible involvement of the thymus in myasthenia gravis has led to the suggestion by Trethewie and Wright[1] that the thymus may influence the formation of acetylcholine (Ach), and by McEachern[2] that it may produce a curare-like substance which interferes with the action of Ach. Experimental evidence concerning the thymus and Ach synthesis is conflicting. Stoerk and Morpeth[3] incubated minced rat brain in Locke solution, with and without added thymoma tissue from a patient who died from myasthenia gravis, and found no significant difference in the amount of Ach synthesized in a three-hour period. Trethewie and Wright,[1] in two experiments, obtained slightly greater yields of Ach when ground brain was incubated with infant thymus than in controls without thymus. In one experiment, using thymus tissue from a person with myasthenia gravis, they obtained a small decrease in Ach formation.[4]

Experiments testing a possible effect of newborn rat thymus on Ach synthesis by brain slices of adult and newborn rats will be reported briefly.

EXPERIMENTAL

In five experiments the brain of an adult rat was split sagittally and each half sliced thinly. The slices of each half were weighed and placed in Locke solution containing glucose 1:500 and eserine 1:5,000. Ground thymus glands from twelve rats less than 24 hours old were added to one lot of slices and both lots

[1] E. R. Trethewie and R. D. Wright, *Aust. and New Z. Jour. Surg.*, 13: 244, 1944.
[2] D. McEachern, *Medicine*, 22: 1, 1943.
[3] H. C. Stoerk and E. Morpeth, SCIENCE, 99: 496, 1944.
[4] See also Torda, C., and H. G. Wolff, *Proc. Soc. Exp. Biol. and Med.*, 57: 69, 1944 for the effect of ether extract of the thymus on synthesis of acetylcholine.

were shaken in an atmosphere of 95 per cent. O_2—5 per cent. CO_2 for one and one quarter hours at 37° C. Two experiments were performed on newborn rat brain in a similar manner, except that slices of six brains were divided in two lots, one for the control and the other for incubation with thymus tissue.

After incubation the total Ach was obtained by treatment with HCl and the extracts assayed on the isolated heart of *Venus mercenaria*. In each experiment a careful comparison of the effects of the extracts of brain tissue incubated with and without thymus tissue was made and the actual amounts of Ach in the extracts estimated by comparison with known amounts of Ach.

RESULTS

The results are given in Table 1. The amount of

TABLE 1

EFFECT OF NEWBORN RAT THYMUS ON ACH SYNTHESIS BY RAT BRAIN SLICES

	Incubated without added thymus	Incubated with added thymus
	Total Ach, γ/gm	Total Ach, γ/gm
A. Adult brain slices		
Exp. 1	10	10
2	6–7	6–7
3	20	20
4	4–6	4–6
5	5	5
B. Infant brain slices		
Exp. 1	3	3
2	1	1

total Ach normally present in whole, adult rat brain is of the order of 1 to 1.5 gamma per gram; that in newborn rat brain of the order of 0.5 gamma per gram. It may be noted, therefore, that appreciable amounts of Ach were synthesized in all experiments. In each experiment, however, the amounts of Ach obtained from brain slices incubated with or without added thymus tissue are the same. It may be concluded that under the conditions of these experiments normal infant rat thymus neither accelerates nor retards the synthesis of Ach by brain slices.

J. H. WELSH
JANE E. HYDE

BIOLOGICAL LABORATORIES,
HARVARD UNIVERSITY

A FIBRINOLYTIC ENZYME IN MENSTRUATION AND LATE PREGNANCY TOXEMIA

THE demonstration[1, 2] that prothrombin and fibrinogen are lacking in menstrual discharge suggests that the blood in it has clotted and the clot dissolved. Evidence for the existence of lytic substances to account for its fluidity has been presented by many but has been inconclusive. Fibrinolytic action in the uterus is nevertheless a rational explanation and a proteolytic enzyme might, in addition, account for the great toxicity of the euglobulin fraction of menstrual discharge, which contains altered protein.[3] Such an enzyme might be a product of endometrial injury due to the withdrawal of hormonal support. Its physiological function might be lysis of damaged tissue, including clotted blood, for the purpose of elimination. The toxic by-product, as has been suggested,[4, 5] might be the final cause of menstruation through vascular injury. Since the hormonal situation in toxemia of late pregnancy is entirely analogous to that at the time of menstruation and the generalized vascular changes similar to the local ones in the menstruating endometrium, we have theorized that this disease might be due to a similar toxin.[5] We have been unable to find such a toxin in the circulating blood at the time of menstruation or toxemia. If a proteolytic enzyme, however, were associated with these states it might be demonstrable, since tests for it are so much more sensitive than our criterion for toxicity, which depends upon obtaining an amount lethal to an immature rat.

A simple method was employed in testing for fibrinolytic activity.[6] Fresh plasma from oxalated human venous blood has been our source of fibrinogen, each sample being tested before use for its ability to form a stable clot with thrombin.[7] Cell and platelet free serum was tested for fibrinolytic activity, always within 24 hours of collection.

Twelve specimens of fresh menstrual "serum" have been examined. All contained marked fibrinolytic activity. A typical protocol is presented in Table 1. There was evidence that the enzyme was even more concentrated in the endometrial "debris" than in the "serum."[8]

During menstruation the venous serum of 5 women

[1] H. D. Glueck and I. A. Mirsky, *Am. Jour. Obst. and Gynec.*, 42: 267, 1941.

[2] E. L. Lozner, Z. I. Taylor and F. H. L. Taylor, *New Eng. Jour. Med.*, 226: 481, 1942.

[3] O. W. Smith and G. V. Smith, *Proc. Soc. Exp. Biol. and Med.*, 44: 100, 1940, and 55: 285, 1944.

[4] G. V. Smith and O. W. Smith, *Am. Jour. Obst. and Gynec.*, 45: 15, 1943.

[5] G. V. Smith and O. W. Smith, *Jour. Clin. Endocrinology*, 1: 470, 1941.

[6] H. J. Tagnon, C. S. Davidson and F. H. L. Taylor, *Jour. Clin. Invest.*, 21: 525, 1942.

[7] The thrombin employed was prepared by the Department of Physical Chemistry, Harvard Medical School, Boston, Mass., from blood collected by the American Red Cross, under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[8] Since prothrombin is lacking in menstrual discharge,[1, 2] the formation of a clot when menstrual "serum" is added to oxalated plasma must be due to the formation of thrombin from the prothrombin of the oxalated plasma and the calcium of the "serum." No clot formed when washed endometrial "debris" was added to oxalated plasma unless calcium or thrombin was also added. Under the latter conditions the subsequent dissolution of the clot occurred with as little as 0.01 cc of "debris."