specific isoagglutinin in the maternal serum is of high titer or if the fetus (or infant) belongs to a compatible blood group. Determining the titer of the maternal isoagglutinins is also of value in the less common cases of suspected hemolytic disease in which the mother is Rh-positive, because here one must still consider the possibility of isoimmunization against factor Hr, or the isoimmunization of an individual of one Rh type against blood of a different Rh type.

The phenomenon of competition of antigens also manifests itself with regard to the different varieties of Rh factors.[7] Of the 3 Rh factors, Rh_o is by far the most antigenic, Rh′ is less antigenic, and Rh″ is the least antigenic.[8] Therefore, it would be expected that if, for example, an Rh-negative woman bears a type Rh_1 fetus and becomes sensitized to the Rh factor, her serum should either contain anti-Rh_o alone, or anti-Rh_o and anti-Rh′ together, but not anti-Rh′ alone. Actual observations fully satisfy these expectations; the cases where the maternal sera appeared to contain anti-Rh′ alone all proved actually to be examples of anti-Rh'_o sera with anti-Rh_c blocking antibodies.[9,10,11] It is highly significant that even though a high percentage of erythroblastotic infants from Rh-negative mothers belong to type Rh_2, only two instances of anti-Rh''_o sera from such mothers have been encountered[12] and none of anti-Rh″ alone, in contrast to the numerous anti-Rh_o sera such cases have yielded. On the other hand, in the far rarer instances of the type Rh_1 mother bearing an erythroblastotic infant of type Rh_2 or type Rh_1Rh_2, as many as 7 anti-Rh″ sera have been observed already, two by the writer[13] and five by British investigators.[14]

These observations on competition of antigens in man may possibly find application in the prophylaxis of hemolytic disease of the fetus and newborn. While specific desensitization of the mother by injections of purified Rh haptens has not proved practicable to date, it is possible that counter-immunization of the mother during pregnancy with a potent but innocuous vaccine may serve to suppress the formation of Rh isoantibodies, and in that way prevent or diminish the severity of the disease in the fetus.

7 For nomenclature, see Wiener, A. S., *Science*, 1944, **99**, 532.

8 Wiener, A. S., *Am. J. Clin. Path.*, in press.

9 Wiener, A. S., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 173.

10 Race, R. R., *Nature*, 1944, **153**, 771.

11 Wiener, A. S., Davidsohn, I., and Potter, E. L., *J. Exp. Med.*, 1945, **81**, 63.

12 Wiener, A. S., and Sonn, E. B., *J. Immunol.*, 1943, **47**, 461; Wiener, A. S., unpublished observations.

13 Wiener, A. S., unpublished observations.

14 Loutit, J. F., personal communication.

14872

Pituitary-Adrenal Cortical Control of Antibody Release from Lymphocytes. An Explanation of the Anamnestic Response.*

THOMAS F. DOUGHERTY, JEANNE H. CHASE,† AND ABRAHAM WHITE.

From the Departments of Anatomy and Physiological Chemistry, Yale University, New Haven, Conn.

The lymphocytes of normal rabbits contain a globulin identical with the normal serum gamma globulin of the rabbit.[1] Labeled globulin, *i.e.*, antibody protein, was demonstrated in lymphocytes obtained from lymphoid tissue of immunized mice.[2] The

* This investigation has been aided by grants from the Josiah Macy, Jr., Foundation, and the Fluid Research Fund of Yale University School of Medicine.

† Alexander Brown Coxe Memorial Fellow.

1 White, A., and Dougherty, T. F., *Abs. Proc. Meetings of Am. Chem. Soc., New York, September*, 1944; *Endocrinology*, in press.

presence of immune bodies in lymphocytes has recently been confirmed in another species, the immunized rabbit.[3] Further, it has been demonstrated that augmented pituitary-adrenal cortical secretion accelerates the rate of release of antibodies from the lymphoid tissue of immunized rabbits.[4] The mechanism of this release is the marked dissolution of lymphocytes which occurs in lymphoid structures within a few hours after increased adrenal cortical secretion.[5] Histological changes in the lymphoid tissue, characterized by lymphocyte dissolution, are reflected in the marked absolute lymphopenia which occurs concomitantly.[6]

Further proof of the role of lymphocytes as a storehouse of gamma globulin and of the hypothesis that pituitary-adrenal cortical secretion controls the rate of release of this globulin, was sought in studies with immunized animals whose sera contained no demonstrable antibodies. The administration of adrenotrophic hormone or of adrenal cortical extracts would be expected to release antibody into the blood at a time when lymphocyte dissolution and lymphopenia are most marked. Moreover, the recognition that a wide variety of unrelated stimuli known to augment pituitary-adrenal cortical secretion[7] are also effective in producing enhancement of antibody titers in the sera of previously immunized animals[8] suggested an explanation of the mechanism of the anamnestic reaction. The enhancement of antibody titer which occurs following the injection of a variety of non-specific substances other than the original antigen has been called the anamnestic reaction.

Experimental. Rabbits and mice were used in this study. The rabbits were males of mixed parentage, approximately 4 months old at the start of the experiments. The mice were of both sexes (NHO strain, Strong) and were 60 to 80 days old at the time the antigen was first injected. The rabbits received commercial chow pellets and oats, and the mice were fed Purina Fox Chow supplemented with calf meal. Food and water were available at all times.

A group of 12 rabbits was injected intravenously on alternate days with 2 ml of a 5% suspension of washed sheep erythrocytes. Agglutinin titers were estimated at intervals on ear vein blood. At the end of 9 weeks the maximum titers obtained were approximately 1-5000. The rabbits then remained in the laboratory for 3 months; at the end of this time no sheep cell antibodies were demonstrable in the serum.

A group of mice was immunized by intraperitoneal injection of 1 ml of a 2% suspension of washed sheep erythrocytes 3 times weekly for 14 weeks. At this time agglutinin titers done on the pooled sera (heart blood) of several groups of 5 animals were 1 to 700. Antigen injection was discontinued and at the end of 4 weeks similar agglutinin titers on 5 groups of 5 mice each showed no circulating sheep cell antibody.

Twelve rabbits and 90 mice prepared in the above manner were used in subsequent experiments. Adrenalectomized mice were used 16 hours after operation. Two types of adrenal cortical extracts were employed, an aqueous preparation (Wilson) and a solution of adrenal cortical steroids in oil kindly supplied by Dr. E. Gifford Upjohn of the Upjohn Company. The desoxycorticosterone acetate was a product of the Schering Corporation. Purified pituitary adrenotrophic hormone (adrenotrophin) was prepared from hog pituitary glands.[9] Potassium arsenite and thiophene-free benzene were commercial products of established purity.

Lymphocyte extracts were prepared as previously described.[2] Agglutinin titers were usually done on lymphocyte extracts and sera using the doubling dilution method. Addi-

2 Dougherty, T. F., Chase, J. H., and White, A., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 295.

3 Harris, T. N., Grimm, E., Mertens, E., and Ehrich, W. E., *J. Exp. Med.*, 1945, **81**, 73.

4 Dougherty, T. F., White, A., and Chase, J. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 28.

5 White, A., and Dougherty, T. F., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 26.

6 Dougherty, T. F., and White, A., *Endocrinology*, 1944, **35**, 1.

7 Sayers, G., Sayers, M. A., Fry, E. G., White, A., and Long, C. N. H., *Yale J. Biol. and Med.*, 1944, **16**, 361.

8 Cannon, P. R., *J. Immunol.*, 1942, **44**, 107.

9 Sayers, G., White, A., and Long, C. N. H., *J. Biol. Chem.*, 1943, **149**, 425.

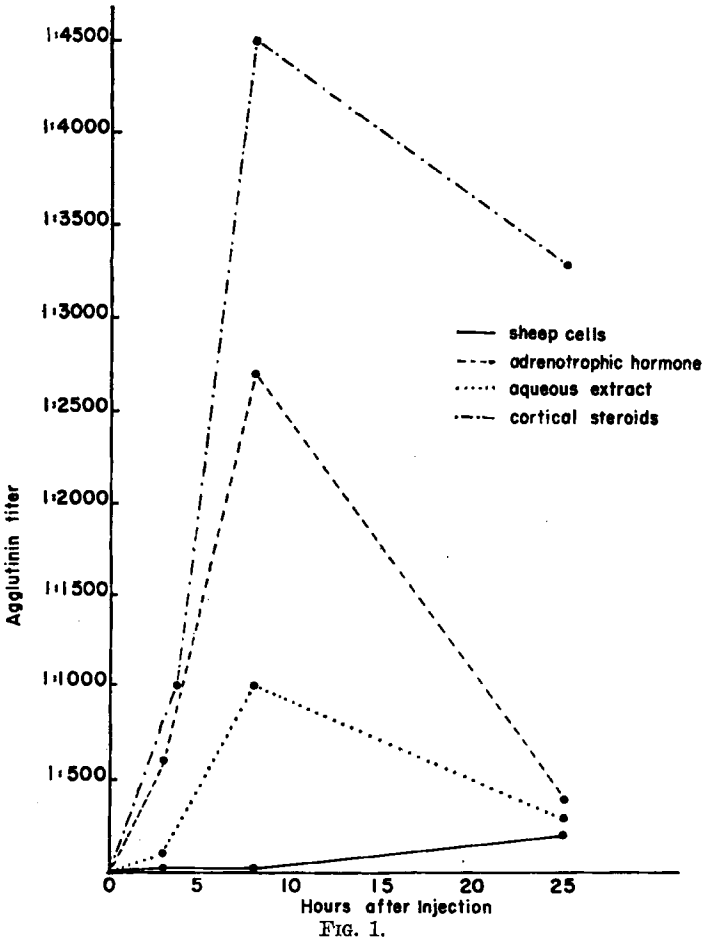

FIG. 1.

Anamnestic response in rabbits. Each curve is the average data for a group of 3 rabbits.

tional dilutions of rabbits' sera were titrated yielding data on dilutions between the intervals of the doubling dilution technic. In the studies with mice each experiment represents the pooled sera and aggregate lymphoid tissue of 5 animals.

Results. The rabbit data which have been obtained are shown in Fig. 1. A single, subcutaneous injection of 5 ml of the oil solution of adrenal cortical steroids produced demonstrable serum agglutinins at 3, 9, and 25 hours after hormone administration (Fig. 1). These were the only intervals at which the bloods were examined. The titers at the 9-hour interval were approximately as high as those which had been reached at the time antigen administration had been discontinued, *i.e.*, maximum. The injection of a single, subcutaneous dose of 10 mg of adrenotrophic hormone in 2 ml water or of 10 ml aqueous adrenal cortical extract also produced a marked release of antibody with the maximal effect evident in the blood at the 9-hour period. In contrast to the definite effect of the 3 types of hormone preparations, a single, intravenous injection of 10 ml of the 5% erythrocyte suspension used as the original antigen produced only a slight anamnestic response. Unpublished results have shown that intravenous injection of sheep cells in the normal rabbit gives evidence of pituitary-adrenal cortical stimulation as manifested by an absolute lymphopenia 3 to 6 hours after the injection.

The data obtained in the experiments with the mice are depicted in Fig. 2. Within 3 hours after the subcutaneous injection of 0.5 ml of aqueous adrenal cortical extract, the titer rose from 0 to 1 to 640 in the non-operated

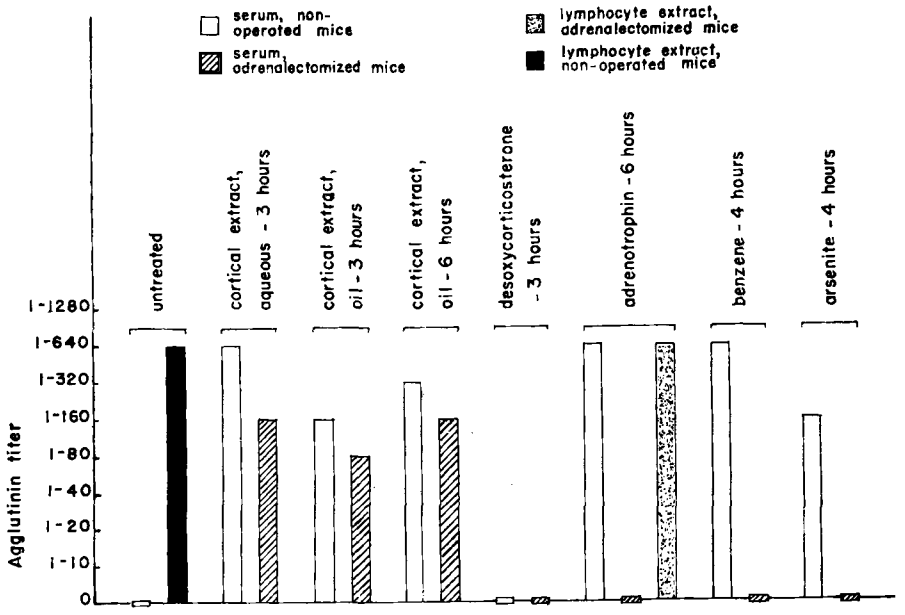

FIG. 2.
Anamnestic response in mice.

animals and from 0 to 1 to 160 in the adrenalectomized group. Within 6 hours after the subcutaneous injection of adrenal cortical steroids in oil, 0.25 ml in the non-operated animals and 0.5 ml in the adrenalectomized mice, antibody titers of 1 to 320 were found in the former and 1 to 160 in the latter. Desoxycorticosterone acetate (1.25 mg in 0.25 ml of oil) was injected subcutaneously into each of 5 non-operated and 5 adrenalectomized mice. No demonstrable antibodies were detected in the pooled sera of either of these groups within 3 hours following the injection. In another group of mice, the subcutaneous administration of adrenotrophic hormone (1 mg in 0.5 ml water) into each mouse produced a titer of 1 to 640 in the non-operated mice within 6 hours. Similar injections of adrenotrophic hormone into adrenalectomized mice did not induce an anamnestic response. Extracts of lymphocytes of these same adrenalectomized mice, treated with adrenotrophic hormone, showed an agglutinin titer of 1 to 640.

Two groups of animals receiving toxic chemical agents were used in order to ascertain whether an anamnestic response could be induced. These substances, potassium arsenite and benzene, have previously been shown to produce adrenal cortical activation, lymphocyte dissolution and lymphopenia in mice.[10] The lymphocyte changes occurred in normal but not in adrenalectomized mice. It may be seen from Fig. 2 that the subcutaneous injection of 0.03 ml of benzene or of 0.1 mg of potassium arsenite (in 0.25 ml water) produced a marked anamnestic reaction in the non-operated animals while none occurred in the adrenalectomized mice.

It should be emphasized that of the animals represented by the data in Fig. 1 and 2 no rabbit nor any group of mice failed to exhibit the anamnestic response as depicted. Data substantiating those in Fig. 1 have been obtained in another group of rabbits in the course of other experiments.

Discussion. The anamnestic response is dependent upon the release of antibody from lymphocytes as a result of pituitary-adrenal cortical stimulation. The presence of gamma globulin in the lymphocytes of normal animals[1] and of labeled globulin in lymphocytes of immunized animals[2] has been demonstrated pre-

[10] Dougherty, T. F., and White, A., unpublished results.

viously. The control of lymphoid tissue size[11,12] and of the numbers of circulating lymphocytes[6] by adrenal cortical hormones is primarily due to the influence of these hormones upon the dissolution of lymphocytes *per se*.[5] Since the dissolution of lymphocytes occurs within a 3- to 6-hour interval following injection of adrenal cortical hormones or of adrenotrophic hormone, the time interval at which antibody globulin appears in the blood following hormone injection should coincide with the retrogressive changes in lymphocytes. This coincidence has been previously demonstrated in hyperimmunized animals.[4] The anamnestic response occurring within 3 to 9 hours in rabbits and mice following a single injection of either aqueous adrenal cortical extract, adrenal cortical steroids in oil, or adrenotrophic hormone further substantiates the relationship between lymphocyte dissolution and globulin contribution to the serum.

The mediation of the effect of the pituitary adrenotrophic hormone by way of the adrenal cortex is seen from the fact that the adrenotrophic hormone elicited no anamnestic response in adrenalectomized mice. However, the lymphocytes of these same mice contained appreciable quantities of antibody. The presence of adrenal cortical steroids is therefore essential for the liberation of antibody globulin from lymphocytes.

The failure of desoxycorticosterone acetate to elicit a response in either the non-operated or the adrenalectomized mice confirms the inability of this steroid to affect lymphoid tissue histology,[10] level of blood lymphocytes,[6] or gluconeogenesis.[1,6,13]

The possibility that antibody might be adsorbed on lymphocytes has been previously considered unlikely.[2] Other investigators have also recognized this possibility.[3] The present data confirm our previous conclusions that antibodies are an integral part of the lymphocyte rather than being adsorbed on the cell from the surrounding medium. Lymphocytes containing considerable quantities of labeled globulin have been obtained from animals with no circulating antibody. It is obvious that since the serum contained no antibody protein, adsorption or absorption of antibody by the lymphocytes could not have occurred. The presence of immune bodies within lymphocytes is not interpreted as evidence that lymphocytes are necessarily concerned with antibody formation.

Many types of stimuli may induce the anamnestic reaction, *e.g.*, non-specific protein injections, hemorrhage, hyperthemia, toxic chemicals, etc. Some of these have been demonstrated to produce pituitary-adrenal cortical activation resulting in lymphoid tissue dissolution, lymphopenia, and an increase in total serum proteins.[10] Of these stimuli which have been studied, benzene and arsenite were very effective in producing the above mentioned alterations in normal mice but to be totally ineffective in adrenalectomized or hypophysectomized mice. Therefore, these two toxic agents were examined in the present study and showed an anamnestic effect in the non-operated mice but not in the adrenalectomized animals. The anamnestic reaction must be based upon the release of antibodies due to the effect of the adrenal cortical steroids on lymphocytes. The data presented further integrate the role of the lymphocyte and of the adrenal cortex in the normal defense mechanisms of the organism.

Summary. An anamnestic reaction has been produced in rabbits and mice following a single injection of adrenal cortical extract or pituitary adrenotrophic hormone. Desoxycorticosterone acetate injection failed to elicit this response. In adrenalectomized mice the anamnestic reaction also was elicited by adrenal cortical extracts but not by adrenotrophic hormone, despite the demonstrated presence of antibodies in the lymphocytes of these animals. Therefore, adrenal cortical mediation is essential for control of the release of antibody from lymphocytes.

Two toxic stimuli, benzene and potassium arsenite, liberated antibodies from lymphocytes in intact mice. These stimuli failed to effect this release in adrenalectomized mice. The data establish the role of pituitary-adrenal

[11] Dougherty, T. F., and White, A., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 132.

[12] Simpson, M. E., Li, C. H., Reinhardt, W. O., and Evans, H. M., *ibid.*, 1943, **54**, 135.

[13] Long, C. N. H., Katzin, B., and Fry, E. G., *Endocrinology*, 1940, **26**, 309.

cortical secretion as the controlling mechanism for the release of antibody from lymphocytes. The anamnestic reaction is one manifestation of this control.

14873

Pyridoxine and Tryptophane Metabolism in Rice Moth Larvæ (*Corcyra cephalonica St.*).

P. S. SARMA. (Introduced by C. A. Elvehjem.)

From the Nutrition Research Laboratories, Indian Research Fund Association, Coonoor, S. India.

Lepkovsky and Nielsen[1] showed that the urine of rats which were kept on a pyridoxine-deficient diet contained a yellow pigment, which turned green at neutral pH on the addition of ferric salts like ferric ammonium sulphate. Later, Lepkovsky, Roboz, and Haagen-Smit[2] isolated the yellow pigment by chromatographic adsorption procedure and identified it as xanthurenic acid. It disappeared from the urine of rats when they were transferred from the deficient diet to one rich in pyridoxine or when pyridoxine was given orally. This compound was first studied by Musajo,[3] who found it in the urine of rats and rabbits fed high protein diets.

It has been shown by the author[4] that pyridoxine is one of the growth promoting factors for the rice moth larvæ (*Corcyra cephalonica* St. Lep.). It was therefore of interest to determine whether the rice moth larvæ excreted any yellow colored compounds in the feces when they were kept on a pyridoxine-deficient diet containing added amounts of tryptophane.

The pyridoxine-deficient diet consisted of salt-extracted wheat flour, sugar, and salt mixture together with the vitamins thiamine, riboflavin, nicotinic acid, and calcium pantothenate in the proportion of 10, 5, 50, and 15 γ per g of diet respectively. Rice moth larvæ which were feeding on whole wheat for a period of 10-12 days after hatching were removed, cleaned, weighed, and placed on the diets given in Table I. The color of the excreta was noted after one week.

These results indicate that on a pyridoxine-deficient diet containing additional amounts of tryptophane, the larvæ excreted yellow-colored feces. Further, it was found that the yellow color disappeared from the feces of the larvæ when the latter were transferred to diets rich in pyridoxine. The same changes in the color of the larval excreta were observed even when bigger larvæ, *i.e.*, larvæ which had fed on whole wheat for 15-20 days after hatching, were placed on the diets mentioned in Table I.

The following experiments were carried out to determine whether the incorporation of tryptophane in the various deficient diets caused the yellow-colored excreta. Rice moth larvæ were taken from whole wheat, divided into 6 equal batches of 20 larvæ and placed on the diets given in Table II.

The results clearly show that only those larvæ which were placed on a diet deficient in pyridoxine excreted yellow-colored feces.

Seven amino acids, cystine, tryptophane, lysine, monohydrochloride, histidine, *l*-leucine, *d*-arginine, and tryosine were fed to the rice moth larvæ at a level of 50 mg of the amino acid to every 5 g of the pyridoxine-deficient diet. It was found that only tryptophane caused the excretion of the yellow-colored feces.

These results clearly indicate that there is an intimate relationship between pyridoxine and tryptophane metabolism in the rice moth larvæ.

The minimum amount of tryptophane

1 Lepkovsky, S., and Nielsen, E., *J. Biol. Chem.*, 1942, **144**, 135.

2 Lepkovsky, S., Roboz, E., and Haagen-Smit, A. J., *Ibid.*, 1943, **149**, 195.

3 Musajo, L., *Chem. Abst.*, 1935, **29**, 6292; *Boll. Soc. ital. biol. sper.*, 1935, **10**, 290.

4 Sarma, P. S., *Ind. J. Med. Res.*, 1943, **31**, 165.