prevention are the result of omission of calcium from or addition of calcium to the diet, respectively. Therefore, since there is complete prevention, in the absence of vitamin D, calcium deficiency *per se* must be regarded as the causative factor. The situation is analogous to that of phosphorus deficiency in experimental rat rickets (Zucker *et al.*,[2] pp. 147-8). Vitamin D cannot fully compensate for very low supplies of the inorganic essential substances (calcium or phosphorus). Only with moderate deficiencies is it possible for vitamin D to improve the net absorption sufficiently to effect prevention. The preventive role of D in gastric lesions produced by moderate calcium deficiency together with other findings in calcium deficiency will be discussed in another report.

Preliminary experiments indicate that the results of Schiödt,[3] who apparently produced exclusively fundus lesions, can be duplicated. No one else has reported such results and we have not seen them until recently. Here the deficiency seems to be one of the B factors as indicated by Schiödt. Further details are now under investigation.

Conclusion. Antral gastric lesions in the rat are produced by calcium deficiency.

2 Zucker, T. F., Hall, L., and Young, M., *J. Nutrition*, 1941, **22**, 139.

3 Schiödt, E., *Acta Med. Scand.*, 1934-35, **84**, 456.

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Complement Fixation Test with Sera of Animals Immunized with Rabies Virus.

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Recently, a reliable technic for complement fixation for diagnosis of rabies was devised by Casals and Palacios.[1] The procedure for preparing antigen is, however, complicated and it seemed desirable to develop a simpler technic for complement fixation which might find application in routine laboratory diagnosis. At first, specific and sharp results were obtained by following the technic developed by Howitt;[2] subsequent experiments showed, however, that further simplification was possible and the following technic was finally evolved, which offers a practical procedure for routine diagnosis.

Antigen. One to 2 g of rabies-infected brain are minced in a Petri dish, spread in a thin layer, and dried in a desiccator over sulphuric acid, at 37°C for 24 hours.* The dried material is scraped off and stored in rubber-stoppered tubes in the ice box. Material thus preserved keeps at least 9 months. For the preparation of antigen, the dried material is ground in a mortar, taken up in neutral physiological saline and added gradually to give a final concentration of 2%. The suspension is centrifuged for 15 minutes at 3000 r.p.m. The supernatant fluid serves as the antigen. This type of antigen can be prepared from the brains of rabbits, guinea pigs, mice, and dogs, infected intracerebrally or peripherally with mouse passage fixed virus, virus from tissue cultures,[3,4] egg passage virus,[5] or with a local strain of street virus. Antigens prepared from brains of guinea pigs and mice infected with equine encephalitis

1 Casals, J., and Palacios, R., *Science*, 1941, **93**, 162; *J. Exp. Med.*, 1941, **74**, 409.

2 Howitt, B. F., *J. Immunol.*, 1937, **33**, 235.

* The preliminary drying is important. Antigens prepared from fresh undried material were found anticomplementary.

3 Webster, L. T., and Clow, A. D., *Science*, 1936, **84**, 487; *J. Exp. Med.*, 1937, **66**, 125.

4 Bernkopf, H., and Kligler, I. J., *Brit. J. Exp. Path.*, 1937, **18**, 481.

5 Kligler, I. J., and Bernkopf, H., *Nature*, 1939, **143**, 899; Bernkopf, H., and Kligler, I. J., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 332.

TABLE I.
Complement Fixation with Brain Antigens from Animals Infected with Rabies and Other Viruses.

Guinea pig antisera obtained by immunization with	Antigens						
	Normal brains	Murine typhus	Equine encephalitis virus	Street virus	Egg passage virus	Tissue culture virus	Brain passage fixed virus
Brain passage fixed virus	—	—	—	+	+	+	+
Tissue culture virus	—	—	n	n	+	n	+
Street virus	—	—	—	+	n	n	+
Equine encephalitis virus	—	—	+	—	n	n	—
Control, normal guinea pig sera	—	—	—	—	—	—	—

+ fixation.
— no fixation.
n not tested.

virus (eastern strain), brains of guinea pigs infected with murine typhus, and normal brains of mice, guinea pigs, rabbits, and dogs served as negative controls.

Sera. Sera of immunized guinea pigs were employed as source of antibody. The animals received a 10% suspension of homologous rabies brain vaccine inactivated with 1% formalin. Three courses of immunization are administered, each consisting of 4 injections of 5 cc, given at 4-day intervals. One group of guinea pigs was immunized with living culture virus and one with a formalin-inactivated strain of street virus. Sera of guinea pigs vaccinated with the virus of equine encephalitis and normal guinea pig sera were used as controls. All sera was inactivated at 56°C for 30 minutes.

Complement fixation test. The test was set up according to Eagle's technic[6] for the Wassermann reaction, using a total volume of 1 cc and 2 units of amboceptor. Our antigens, even in the highest concentration, did not exhibit anticomplementary reactions. Fixation was carried out for 16-18 hours in the ice box, followed by 30 minutes at room temperature. Water bath fixation at 37°C was not sufficiently sensitive. After addition of the hemolytic system, the mixture was incubated for 30 minutes in a 37°C water bath and the results were read as soon as tubes containing antigen and serum controls became clear.

Results. Typical results are summarized in Table I. Anti-rabies immune sera produced by the procedure outlined gave specific results and failed to react with brain antigens of animals infected with typhus or equine encephalitis or of normal brains.† Normal sera were always negative.

Comparative experiments with our strains of street and fixed virus yielded the following results:

Antisera produced by immunization with street virus (local strain), fixed complement in lower titers than did sera of animals immunized with fixed virus, when either fixed virus or street virus brain was employed as test antigen. The latter sera reacted up to a dilution of 1:400 with standard antigens prepared from brain of mice infected with fixed virus. When brains of dogs infected with our strain of street virus were employed as antigen, the titers of these sera were lower. Thus, sera with a maximum titer of 1:125 failed to react with brains of dogs infected with street virus; positive results were obtained with this antigen only when sera of a titer of at least 1:250 were employed. This may perhaps be due to a lower concentration of antigen in dog brain than in mouse brain. When different parts of dog brains were tested for their antigen content, considerable differences were observed; the highest titer was always found with medulla which gave reactions when diluted 1:16, while cortex, midbrain, and cerebellum

[6] Eagle, H., *The Laboratory Diagnosis of Syphilis*, 1937, C. V. Mosby Co., St. Louis.

† Satisfactory antigens were obtained from partly decomposed brains of animals which had died of rabies.

gave consistently lower titers.

The technic used for the preparation of rabies antigen proved unsuitable for the preparation of antigens from brains of animals infected with equine encephalitis virus. It appears that simple desiccation is detrimental to this antigen. Frozen brains dried in a vacuum yielded antigens which reacted with anti-equine encephalitis sera but exhibited marked anti-complementary properties.

The rate of appearance and the titer of neutralizing and complement fixing antibodies respectively, were observed during a period of 4 months in a group of 5 guinea pigs which had received one course of immunization against rabies. The complement fixing antibodies reached a maximum titer about a week after the last injection, decreased to 1/8 of the maximum titer after one month and disappeared completely after 4 months. The neutralizing antibodies, however, reached a maximum titer after about one month and did not show a decrease during the subsequent period of observation. The results indicated a lack of correlation of complement fixing with virus neutralizing antibodies in rabies, and are in accord with the findings of Casals and Palacios.

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Effect of Growth Hormone on Glycosuria of Fed Partially Depancreatized Rats.*

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Although it has been well established that the secretions of the anterior pituitary profoundly influence carbohydrate metabolism, it has not been shown as yet which hormone or hormones are involved in this relationship. This problem has recently been comprehensively reviewed by Houssay.[1] Heretofore investigators have used whole pituitary extracts or hormone preparations only partially purified. Recently much progress has been made in the isolation of the anterior pituitary hormones. We have available at present the following hormones in a state which approaches physiological purity: growth, lactogenic, interstitial cell stimulating, and adrenocorticotropic.

A study is being carried out to determine which of the hormones of the anterior pituitary will produce glycosuria in the partially depancreatized rat. The data presented here have to do only with the effect of growth hormone upon the glucose excretion of such an animal.

Methods. The test animal used in these experiments is a potentially diabetic male rat in which a partial pancreatectomy had been performed 5 to 6 months previously, when the animal was 4 to 5 weeks of age. The rats were fed a standard diet except for the 24-hour periods in which the glycosuria was measured. During this period, the animals were placed in individual metabolic cages, and given 75 cc of a 20% sucrose solution as their only food and drink. All animals but one operated and one normal drank the total amount. Under these conditions it was found that the partially depancreatized rat excretes from 0.1 to 4.0 g of glucose in 24 hours. The unoperated rat of the same age excretes as a rule less than 0.1 g of glucose during 24 hours.

The growth hormone preparation used was a "cysteine treated globulin fraction," prepared as reported recently,[2] which had a potency of approximately 23 growth hormone units per milligram (hypophysectomized rat

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1 Houssay, B. A., *Endocrinology*, 1942, **30**, 884.

2 Marx, W., Simpson, M. E., and Evans, H. M., *J. Biol. Chem.*, 1943, **147**, 77.