have transmitted the eastern disease to guinea pigs, and in one instance the bite of one *Aëdes aegypti* transmitted the western virus to a guinea pig. Three other insects of the same lot, each fed upon a different animal, failed to infect. The virus appears to persist in at least some of the mosquitoes as long as they live. Eastern virus has been transmitted by *Aëdes sollicitans* 33 days after the infective meal, the longest period we have been able to keep a sufficient number alive for test. The longest period we have found *Aëdes aegypti* capable of transmitting the western disease is 63 days. Virus was shown to be present in this same lot of mosquitoes 93 days after feeding, but they did not transmit the disease at this time.

MALCOLM H. MERRILL
C. WM. LACAILLADE, JR.
CARL TEN BROECK
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, NEW JERSEY

MECHANISMS IN THE DEVELOPMENT OF AN ACTIVE RESISTANCE TO THE EFFECTS OF SUBSTANCES STIMULATING THE THYROID GLAND IN THE GUINEA PIG

IF by means of extirpation of considerable portions of the thyroid, growth processes leading to compensatory hypertrophy are initiated in the guinea pig, simultaneous administration of KI intensifies these proliferative changes.[1] Similarly, if KI is either given orally to or injected intraperitoneally in certain quantities into guinea pigs or rats with normal thyroids, a marked increase in mitotic proliferation as well as a slight increase in the size of the acinus cells of the thyroid and a moderate softening of the colloid, accompanied by the invasion of this latter substance by phagocytes, can be observed.[2] These changes reach a maximum within a certain time, which varies according to the mode of administration of the iodine salt and differs in guinea pig and rat. When injections are continued following this period of maximum effect, a decrease in the mitotic activity occurs; the gland returns to the normal or perhaps to a subnormal level of activity; at the same time the acini may become distended with colloid.

Corresponding observations can be made, if instead of iodine salts we inject optimal quantities of extracts of anterior pituitary glands of cattle into guinea pigs; however, in this case the effects are much greater. There is an extraordinary increase in mitotic proliferation of the thyroid; the acinus cells increase very much in size and the colloid is largely liquefied and absorbed; thus the whole gland changes its structure and becomes similar to the thyroids seen in very pronounced cases of Graves' disease.[3] Furthermore, it is possible by these means to imitate the principal functional symptoms of this disease. But in this case also, after a stage of the maximum effects has been reached, a return of the thyroid gland to its normal state may take place gradually, notwithstanding the continued injections of the extract;[4] in a parallel way a decrease in the functional and metabolic hyperactivity of the gland sets in.[5] Similar observations were recently recorded by Collip and Anderson in the rat[6] and by Hertz and Kranes in the rabbit.[7]

As to the mechanism underlying this process of retrogression, we tested about three years ago the ability of the blood serum of guinea pigs, which had become resistant to the effects of anterior pituitary extracts, to neutralize these extracts *in vitro;* we made mixtures of such blood serums and extract and injected them into fresh guinea pigs. In control experiments we injected mixtures of normal guinea pig serum with anterior pituitary extracts. The results of these experiments were negative (see footnote 3-c). However, in their recent experiments, Collip and Anderson succeeded in demonstrating that the serum of rats which had become refractory to extracts was able not only to prevent the rise in metabolism otherwise caused by the injection of the thyroid stimulating hormone of anterior pituitary, if serum and extract were mixed *in vitro* previous to injection, but even to lower the basal metabolic rate of the injected animal.[8] We may therefore conclude that the development of substances neutralizing the thyroid stimulating hormone of the anterior pituitary gland and circulating in the blood of the injected animals is one of the mechanisms underlying the acquired resistance to the effects of the extract.

But we believe that there are reasons for assuming

[1] Leo Loeb, *Jour. Med. Research,* 40: 199, 1919, 41: 481, 1920; *Am. Jour. Path.,* 2: 19, 1926; 5: 71 and 79, 1929. S. H. Gray, *Am. Jour. Path.,* 5: 415, 1929. Elizabeth Moore, *Archives of Path.,* 16: 657, 1933.

[2] (a) S. H. Gray and Leo Loeb, *Am. Jour. Path.,* 4: 257; (b) I. Rabinovitch, *Am. Jour. Path.,* 4: 601, 1928; 5: 91, 1929; (c) *Proc. Soc. Exp. Biol. and Med.,* 28: 394, 1931.

[3] (a) Leo Loeb and R. B. Bassett, *Proc. Soc. Exp. Biol. and Med.,* 26: 860, 1929; (b) 27: 490, 1930; (c) Leo Loeb, *Klin. Wochensch.,* No. 51 and 52/53, pp. 2121 and 2156, 1932.

[4] Leo Loeb and Hilda Friedman, *Proc. Soc. Exp. Biol. and Med.,* 29: 172, 1931; Leo Loeb, *Klin. Wochensch.,* Nos. 51 and 52/53, pp. 2121 and 2156, 1932.

[5] (a) W. J. Siebert and R. S. Smith, *Proc. Soc. Exp. Biol. and Med.,* 27: 622, 1930; *Am. Jour. Physiol.,* 93: 396, 1930; (b) W. J. Siebert and E. W. Thurston, *Proc. Soc. Exp. Biol. and Med.,* 29: 652, 1932.

[6] M. B. Collip and E. M. Anderson, *Lancet,* 226: 76, 1934.

[7] S. Hertz and A. Kranes, *Endocrinology,* 18: 415, 1934.

[8] M. B. Collip and E. M. Anderson, *loc. cit.*

that in addition to this mechanism also other mechanisms may play a rôle in this process of immunity. The following facts seem to us to favor such a conclusion. (1) The curve representing the rise and fall in the mitotic proliferation in the guinea pig thyroid glands shows a maximum in the first few days of the injections and then declines at a time when neutralizing substances can not yet have developed (see footnote 3-a). (2) The curves representing the first stage of increasing stimulation and the following stage of decline are in principle similar in the case of the anterior pituitary hormone and of iodine preparations acting as stimulants. It appears improbable that the acquired resistance to the effects of iodine is due to the development of iodine-neutralizing substances, able to act *in vitro*. Moreover, in the rat the maximum stimulation is reached as early as five days following the beginning of the injections and then a rapid decline sets in, at a time therefore too early for the development of neutralizing substances (see footnote 2-c). (3) There is known already a mechanism counteracting the thyroid stimulating effect of anterior pituitary hormone and initiated through the injection of anterior pituitary extract which is not dependent on the action of substances neutralizing the hormone *in vitro*. It consists in an increase in the production of thyroid hormone. The latter tends to lower the level of reactivity of the thyroid gland to all stimuli so far tested, such as the removal of a great portion of the thyroid gland,[9] administration of KI[10] and injection of anterior pituitary extract.[11] (4) There is reason for assuming that the neutralizing substances develop in response to the injection of a protein from which the thyroid stimulating hormone of cattle anterior pituitary could not be separated so far; this protein is foreign to rat or guinea pig. We may tentatively assume that a less complex chemical group attached to this protein functions as the hormone proper. An excess of the animal's own thyroid stimulating hormone would therefore not lead to the production of a hormone-neutralizing substance, although it may activate other antagonistic mechanisms, such as the one mentioned under No. 3. (5) In the case of mouse tumors it has been possible to show that the relative immunity, which develops against the action of substances inhibiting tumor growth, such as colloidal metals and hirudin, depends upon a combination of two processes, one residing in the organism and a second one developing in the affected tumor cells themselves.[12] It is probable that we have to deal with a similar combination of mechanisms also in the case of the thyroid-stimulating hormone of the anterior pituitary. We may assume that the change which takes place in the stimulated acinus cells of the thyroid gland alters their response to the subsequent application of the same kind of stimuli. The intensity of reaction must necessarily decrease in the course of continued application of stimuli; otherwise the results of stimulation would accumulate in geometric progression.

Retrogressive changes during long-continued injections of anterior pituitary extracts are not limited to the thyroid gland; the changes produced in the ovary of the guinea pig also disappear and at about the same time as those in the thyroid. In this connection it may be stated that in experiments with Hilda Friedman, we have found it impossible so far to separate the stimulating effect of the anterior pituitary on the thyroid from the effects of this substance on the ovary of the guinea pig. The latter consist in atresia of the follicles, in the formation of interstitial gland and of pseudocorpora lutea of theca interna origin (see footnote 3-b). The term "thyrotropic" hormone does not therefore seem suitable for a substance which invariably also exerts "gonadotropic" functions. The term "thyroid stimulating" characterizes this hormone sufficiently without implying that it is specifically "thyrotropic."

LEO LOEB

WASHINGTON UNIVERSITY
SCHOOL OF MEDICINE,
ST. LOUIS

[9] Leo Loeb, *Jour. Med. Res.*, 41: 481, 1920.

[10] S. H. Gray and I. Rabinovitch, *Am. Jour. Path.*, 5: 485, 1929.

[11] Leo Loeb, R. B. Bassett and Hilda Friedman, *Proc. Soc. Exp. Biol. and Med.*, 28: 209, 1930.

[12] M. S. Fleisher, Miguel Vera and Leo Loeb, *Jour. Exp. Med.*, 20: 522, 1914; M. S. Fleisher and Leo Loeb, *Jour. Exp. Med.*, 21: 155, 1915.

RETENTION OF CARBON DIOXIDE GAS IN THE INTERCELLULAR ATMOSPHERE OF PEARS AND APPLES

THE use of carbon dioxide other than as a refrigerant offers many possibilities in the field of horticultural science. The recent work of Brooks *et al.*[1] has indicated its value in preventing fungal infection and in the retardation of the respiratory processes during transportation of perishable fruit and vegetables. Kidd and West[2] have shown that in the gas storage of apples under certain controlled conditions carbon dioxide markedly increased the storage life of the fruit. Results obtained by Harley and Fisher[3]

[1] C. Brooks, E. V. Miller, C. O. Bratley, J. S. Cooley, P. V. Mook and H. B. Johnson, "Effect of Solid and Gaseous Carbon Dioxide upon Transit Diseases of Certain Fruits and Vegetables," *Tech. Bul.* 318, U. S. Dept. Agr., 1932.

[2] F. Kidd and C. West, "Gas Storage of Fruit. III. Lane's Prince Albert Apples," *Jour. Pomol. and Hort. Sci.*, 11: 149, 1933.

[3] C. P. Harley and D. F. Fisher, "A Study of the Internal Atmospheres of Apples in Relation to Soft Scald," *Proc. Amer. Soc. Hort. Sci.*, 271, 1930.