Thirty first meeting.

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President Lee in the chair.

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Reply and explanation to recent criticism of my experimental study on effects of extirpation of the salivary glands on the gastric secretion.

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It is not always a congenial task to have to reply to a criticism of one's experimental work. To many a conservative thinker, the policy contained in a remark attributed to Ludwig under a similar circumstance, "Schweigen ist gold," may appeal as more expedient. But yet, the dignified silence may be interpreted, by the one who has advanced the criticism and even by the research worker and general student of physiology, as a tacit approval to the fault finding — in other words, as signifying that the criticism was deserved and the work criticised defective. I find myself in this embarrassing position with regard to an article published in the "Proceedings of the Society for Experimental Biology and Medicine, 1908, v, pp. 114–117," New York, by Dr. A. S. Loevenhart and Dr. D. R. Hooker, entitled: "Note on the supposed presence of a gastric hormon in the salivary glands."

Although the physiology and pathology of digestion has been my life work, yet, as one of the results of many years of laboratory teaching and training, I am loathe to insist dogmatically on any of my opinions and am ready at any moment to be corrected and to advance another step in the attainment oftruth. ("Experientia fallax, Experimenta mendax.")[1]

[1] But rather than dwell upon the moral side of scientific controversy I prefer to refer to Sir Thomas Browne's "Religio medici," 1904 edition, p. 98.

Especially welcome are such corrections when they emenate from such an esteemed friend and talented worker as Dr. Loevenhart. The original worker whose results are criticised has the right, however, to demand that his special point of inquiry ("Fragestellung") and all the methods of experimentation, operative, physiologic and chemic, shall be conscientiously repeated on, at least, an equal number of the same kind of animals, successfully nursed through the identical operative procedures. He has a right to demand a scrupulous regard for detail, and for all the finer distinctions made in his application of methods, some of which may have required years for their perfection in his hands and those of his associates.

Let us investigate whether my friend, Dr. Loevenhart, has fulfilled these indispensable, fundamental conditions that should precede destructive criticism.

I sought to ascertain the effect of salivary gland extract in dogs deprived of all four pairs of salivary glands, whose gastric juice had been carefully studied before any operation of removing the glands was undertaken. Sometimes the removal had no very marked effect; but in those dogs in which it did, I tried to ascertain whether the depressed gastric secretion could be restored or not by salivary gland extract. I tried to study the effect on a secretion already abnormally depressed in three series of dogs — thoroughly recovered from the operation, allowing ten days to two weeks, at least, for recovery.

Dr. Loevenhart starts with normal dogs, as he supposes, and expects to raise the gastric secretion qualitatively and quantitatively *above* the normal. He seeks the effect of salivary gland extract in raising a supposedly normal gastric secretion to a higher acidity and proteolysis — an entirely different problem from mine.

I have never published anything on the effect of salivary gland extract on the normal gastric secretion of dogs. It is not asserted that this extract can raise the gastric secretion *above normal*, but only that it may, under certain conditions, partially restore a gastric secretion that is depressed *below* normal. Dr. Loevenhart is attempting to change a normal secretion to an abnormal (higher) one. I studied the effect in restoring an abnormal secretion to a normal one.

When there are four different procedures for obtaining gastric juice on the same dog within thirty minutes, and the jugular vein exposed, a cannula inserted and submaxillary extract injected intravenously, it must not be overlooked that, with every additional interference, the animal becomes more and more disturbed and that this seriously influences his gastric secretion. The chemico-physical and the neuro-physical processes of secretion are thoroughly upset unless a long time for recovery is given. This is shown in Dr. Loevenhart's results, page 4 of his reprint, in which the total acidity and free HCl and the proteolytic power became less and less in specimens *A*, *B* and *C*; only when the psychic secretion was aroused, granting that this was not a delayed effect of injection sal. gl. extr. specimen *D*, was there any notable proteolysis without addition of acid. The notes of the beginning of experimentation on this dog bear the date of April 6, and the qualitative studies bear the date of April 8 — not near time enough to permit dog No. 2 to entirely recover.

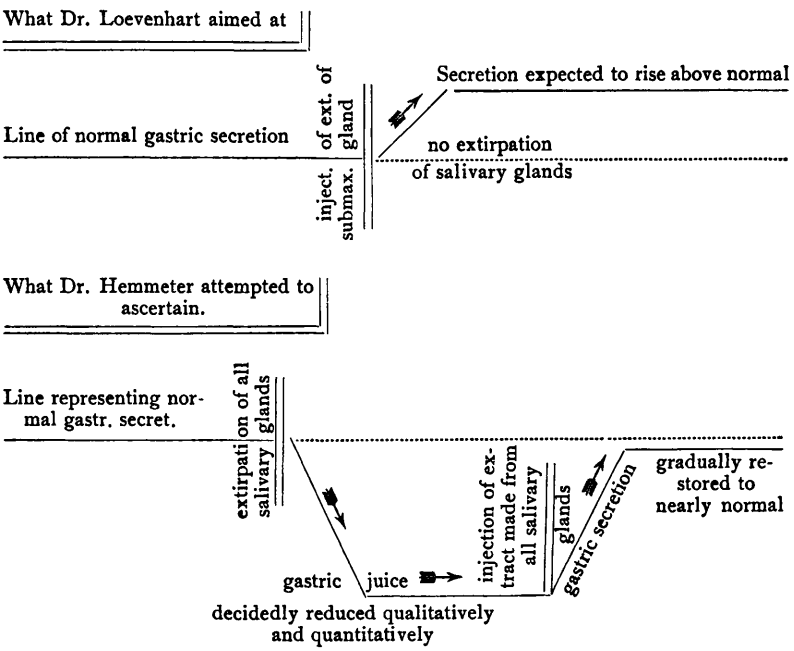
To expect salivary gland extract to raise the gastric secretion qualitatively and quantitatively above what is the regular standard for the average dog is to expect something abnormal — for an unusually abundant and unusually active gastric juice is logically as abnormal as one that is unusually diminished or inactive.

1. What Dr. Loevenhart presumes is that the salivary gland extract should change a normal gastric juice to an abnormal one (from the regular amount to an unusually high amount and activity).

2. What I attempted to ascertain was whether or not an abnormal gastric juice could be restored to the normal (from diminished and weakened secretion to the normal). The "Fragestellung" is not the same, in fact, it is highly digressing.

Dr. Loevenhart observed only two animals. Nowhere does he give the date of operations, nor state the time that elapsed between the operation and first day of experimentation, nor the amount of proteolysis in millimeters of Mett tubes. Both animals were abnormal. The first dog, No. 1, he admits had distemper and was feverish, was thin and would not eat. The first observations are dated November 11, 1907, and this animal died within 48 hours.

All of our results were gained from dogs that lived for three to six months and then had to be killed in most cases because we had no room or facilities for keeping them during the summer vacation — excepting the series of the summer of 1907 when I kept four dogs at our country home.



Dog No. 2 of Dr. Loevenhart was also abnormal. This is evident from the feeble proteolysis as indicated by Mett tubes (in Dr. Loevenhart's article they are called "Metz" tubes) and the low acidity, and Dr. Loevenhart gravely states that the fluid gained by catheterization, 8.6 c.c., specimen *A*, contained *much dark mucus* (blood? and mucus). Mucus in a fasting dog's stomach is one of the most reliable indications of gastritis. Dog No. 2 had a diseased stomach also.

We made our salivary extract from maceration of all four pairs of canine salivary glands, even the orbital — and it is all important that this extract should be made only from salivary glands that have been functionally active immediately before their excision

(the dog must be made to chew bread and then rapidly etherized). I have worked with extracts of inactive glands, but so far have refrained from publishing anything concerning their effects or non-effect. In not a single instance, has the operative plan and technique used by us, nor the physiologic routine of preparing the glands by functional work, nor the chemic discipline of ascertaining the proteolytic activity been punctiliously carried out by Doctors Loevenhart and Hooker.

Both of the animals had diseased stomachs. In neither were the salivary glands extirpated. The entire plan of experimentation and aspect of physiologic inquiry is so fundamentally different from mine, that comparison of their work with ours is not logical, and any deductions from their work as used to interpret our results are unfortunately misapplied.

It is only fair that the work of an experimentor should be judged from his most recent publication, in this case that which appeared in the *Biochemische Zeitschrift* (Hamburger Festschrift, Band xi, p. 238), the only complete report published by me.

The short notice by which Drs. Loevenhart and Hooker judged our work was nothing but a preliminary report, and contained, as such reports occasionally do, some inaccuracies which I have taken the privilege to correct in the article published in the *Biochem. Zeitschr., l. c.* ("Die Wirkung der Total Extirpation Sämtlicher Speicheldrüsen auf die Sekretorische Funktion des Magens beim Hunde"). Even in this article the printer has allowed some wrong figures to slip into the headings of tables C, D and F, pp. 257, 258 and 259, for which I am in no way responsible, but which do not injure the main argument, especially as the editors of the *Biochem. Zeitschr.* politely corrected them in a subsequent *Berichtigung*.

In Dr. Loevenhart's experiment on April 8, submaxillary extract was injected into dog No. 2 at about 3.10 to 3.15 P. M., the gastric juice of twenty minutes later showed a free HCl of 0.20 (titration with *n*/20 NaOH) but the proteolytic power with addition of acid is declared to be "*good.*" But at 3.30 the stomach of the same dog was catheterized and specimen *A* obtained after the dog was allowed to smell meat for ten minutes. This specimen *A* was the most active that Loevenhart obtained. It came 35

minutes after the submaxillary extract was injected. Question is: Would not this active juice have been secreted even without the efforts to cause a psychic secretion, for the salivary extract in my experience has a latent period in which it produces no very marked secretion? After that period it may come; that is, a pronounced secretion may come, even thirty minutes after injection of salivary extract and even if there has been no chance for psychic secretion. Pawlow, *l. c.*, p. 70, states that in all cases the latent period after the vagus stimulation of gastric secretion may be from 15 minutes to one hour, and even more.

Hitherto we have known the term "*latent period of secretion*" only in connection with the stimulation of a nerve going to gland or muscle. We are not so familiar with the use of the term "latent period" in connection with the chemical stimulation of a gland. A moment's reflection will bring the thought nearer to us that even after nerve stimulation, pure and simple, chemical events must transpire in the gland cells which require a certain time for their elaboration. Now, if the stimulation is purely chemical, and not through a nerve, the same or similar chemical events must precede the actual outpouring of secretion. We are still ignorant of the processes that occur during the "latent period," but recent work indicates that they are partially electrical and partially of a chemical nature. We must also consider that the immediate effect of a chemical stimulation, like the immediate effect of a nerve stimulation, may be inhibited.

There are so many side influences of a physical, nervous and chemical nature which control the phenomenon of the "latent period" that its exact nature and what transpires during it, is still a matter of speculation.

It may, at first sight, seem paradoxical that the latent period of secretion after sham feeding in dogs is stated by Pawlow to be only 5 to 10–15 minutes, and the latent period after vagus stimulation 15 minutes to one hour — for in both instances the stimulation is transmitted by one and the same nerve to the identical synapses in the gland cells. Pawlow explains this, p. 71, *l. c.*, by his belief that in artificial stimulation of the vagus, the stomach receives the excitatory as well as inhibitive impulses, and the latter check secretion.

How can we conceive of inhibitive processes to explain a long latent period of secretion, when chemical substances (for example, salivary gland extracts) are injected intravenously? By an analogous experimental reasoning, we have learned (Pawlow, *l. c.*) that it is impossible to imitate the influence and action which the vagus exerts during normal life while digestion is going on, for our laboratory methods are far too coarse and the complexity of fibers in this magnificent highway of nerve tracks too intricate for us to single out individually functioning secretory fibers.

We are not much better off when we attempt to imitate the chemistry of the internal secretion of glands, for only in a single instance has a hormon been isolated in a state that reveals its exact chemic structure.

The chemic messengers are bodies of definite chemic structure which are released with unerring exactness from their producing organs; but when we manufacture an organ extract, it is, of course, possible that we may seize the hormon (if I may still use the term); but unavoidably we must extract the entire tissue of the organ and as a result obtain extracts, which contain materials that stimulate, but also materials that may inhibit secretion. This occasional inhibitive effect of salivary gland extract on gastric secretion has brought to mind two ideas: either that I am not dealing with a hormon or stimulator at all, or that there may be two kinds of chemic correlation, one that stimulates and the other that inhibits. The conception which sees an antagonistic, as well as a synergistic, correlation brought about by chemic messengers is at least as rational, when applied to the physiologic correlation of organs by means of chemic substances communicated to them by means of the circulation, as when applied to the correlation of organs by means of nerve elements. This relation of organs by means of reciprocal (antagonistic or synergistic) action of nerves is not new to physiologists, and has been brought home to us in a most impressive manner by Meltzer, not to mention Ch. S. Sherrington, New York, 1907. All of this is still hypothesis; but this hypothesis has been given color (1) by the seemingly paradoxical effects of (*a*) such a pure substance as adrenalin, which does not always cause constriction of vessels (only when they are severed from the nerve centers) but sometimes may cause dilatation, when in normal animals a certain vascular area is intact in connection

with its nerve centers,[1] and of (*b*) gland extracts, which sometimes raise blood pressure and often lower it (sometimes after a slight previous rise), and (2) by the contradictory effects of some salivary gland extracts on gastric secretion. All of this doubt will continue so long as we are compelled to deal with a complex mixture of various substances in gland extracts and not with one pure substance of known composition.

To this consideration belongs, also, the antagonistic phenomena reported by Lilienfeld, Morowitz and Delezenne as occurring in blood coagulation (positive and negative phase of coagulation). This is explained by Lilienfeld and also by Delezenne by the isolation from blood plates and leucocytes of two substances, one of which they term "*leuconuclein*" which favors coagulation, and the other, "*histon*," which retards coagulation. Before the isolation of these two substances the phenomenon of the positive and negative phase during blood coagulation appeared paradoxical, and the idea of a **latent period of coagulation** might have come to many an experimentor. Just so with the latent period after chemical stimulation of the glands; it may be due to inhibitive substances in the gland extracts used, and it is possible that this delay in bringing about the effect after chemical stimulation of the gastric glands, may disappear with a clearer knowledge of the chemistry of the gland extracts, and a more accurate method of preparing them.

Besides the latent period of secretion, we must consider the neutralization of the first acid secreted by the mucus present in the stomach. Pawlow ("Arbeit. d. Verdauungsdrüsen," *l. c.*, p. 39) calls attention to what he emphasizes as "Factum," namely, "*Even with a normal stomach and with a pure gastric juice 25 per cent. of its acidity can be lost through neutralization by mucus.*" How much more must this neutralization take place in a stomach that, as Loevenhart states, gave "*much dark mucus.*" The very efforts of catheterization increases the mucus formation, and after the submaxillary gland extract was injected, if it had any stimulating effect at all (I am not prepared to state whether it had or not) this much is sure, the mucus had to be neutralized before

[1] This latter effect of adrenalin is not a purely chemical effect but a mixed effect of nerve and chemical phenomena. One and the same chemically pure substance cannot be claimed to contain both stimulating and inhibitive substances.

there could be free HCl. The extract was injected at 3.20 on April 10; at 3.25 P. M. the gastric juice was drawn by catheterization (8.8 c.c., specimen *C*). No free HCl was in it, but six minutes after the injection of salivary extract the dog was shown meat, and ten minutes after that there was a fourth catheterization (the fourth in 30 minutes). This 5.3 c.c. was active juice and Loevenhart and Hooker attribute it to psychic secretion.

Considering the latent period of secretion and the time for neutralization by mucus, it is reasonable to inquire whether or not the injection of extract had a feeble but delayed influence, although Loevenhart and Hooker used only submaxillary extract and not that of all four pairs of glands, and did not prepare it in the manner I did.

Concerning the inflammation (gastritis) in the stomachs of their dogs, I can very readily appreciate the difficulty, for I had been thwarted and misled by diseased canine stomachs for almost a year before we gradually learned to recognize, avoid and treat them.

Evidences like these, naturally suggest that such experiments cannot be successfully carried out in a few months. I was not aware of Dr. Loevenhart's criticism, until November 14, 1908. That there are salivary extracts that have no peptogenic effects whatever, and others that are variable, I have already stated in my article in the *Biochemische Zeitschr.*, Vol. xi, p. 251 ("Verschiedenheiten in d. peptogenen Kraft d. Speicheldrüsen Extrakten").

Then again, the complexity of the mechanism of gastric secretion in dogs is such (*Biochem. Zeitschr., l. c.*, p. 253) that the initial depression caused by extirpation of the salivary glands probably may be gradually replaced by special efforts of the remaining sources of stimulation to the gastric glandular apparatus.

This problem is far too deep and complicated to have years of laborious experimentation set aside by a casual testing of two sick dogs, as to whether a saline extract of the inactive submaxillary gland alone can cause a secretion of gastric juice in animals not deprived of their salivary glands.

That there may be defects in my work I am willing to accept as a possibility, because a general knowledge of the history of physiology reveals the status that the first results of similar ex-

perimental work are only in most exceptional instances without defects or errors.

Such a defect in the connexus of cause and effect has recently been brought to my knowledge and, today, makes it debatable whether the name "hormon" is correctly applied by myself to the stimulating quality of one gland extract upon the secretion of another set of glands. The definition and conception of the hormon allows a rather wide application, it is true, but it seems to me it ought to be restricted to substances whose chemical structure is at least approximately known and that have one predominant characteristic or specific effect on other glands, in which effect they cannot be replaced by extracts from other organs or tissues. This is not the case with the salivary extracts, for, as we can learn (*Biochem. Zeitschr.*, Vol. xi, p. 253), extracts of the pyloric mucosa and of the spleen (Luciani) act in a similar manner in stimulating gastric secretion.

Concerning the pepsinogenous effect of the spleen on the gastric secretion, I refer to the work of Tarulli and Pascucci, executed in Luciani's laboratory and described in the latter's splendid work, "Physiologie des Menschen," translated into German by Baglioni and Winterstein, Vol. ii, pp. 151 and 152. On page 153 it will be seen that the extract must be made from an *active* spleen, as Luciani says "a spleen that is hyperemic and swollen," which means, taken from a dog during the height of the digestive period. Extracts of spleen taken during the period of functional rest had no pepsinogenous effect; but the meaning of Luciani and his pupils above mentioned is unmistakable. A chemical substance is formed in the spleen during its activity which, when brought into the circulation, is absorbed by the gastric glands and is capable of augmenting the quantity of the secreted pepsin. Additional emphasis is given in these experiments to the fact that the extract should only be made from a functionally active gland.

Whatever may be the final outcome of investigations concerning the chemical nature of the hormones, Bayliss and Starling consider that they were originally accidental by-products of the activity peculiar and proper to the organ which has produced them. Thereafter the next step in the development of a correlation is the acquisition of a sensitiveness or a responsiveness to the hormones

in any remote organ ("Die Chemische Koordination der Funktionen des Körpers," *Ergebnisse der Physiologie*, Jahrgang v, p. 670). The only word to which I could take exception in this explanation of Bayliss and Starling is the word "accidental" ("*Zufällige*" Nebenprodukte). I should like to enlarge this conception when applying it to the digestive tract, and state that the various segments of the digestive tube are correlated and coordinated by a sensitiveness not *only to accidental products*, but to the regular by-products which are known to accompany the formation of the specific products of the organs of digestion.

An infirmity in the experimental logic, suggestive of a metabolic by-product produced in the salivary glands during activity which might be regarded as a chemical messenger to the secretory apparatus of the stomach, might be found in the occasional failure to produce total loss of gastric secretion after the salivary glands are removed. In other words, we should expect to find invariable "*Ausfalls-Erscheinungen*," phenomena of lapse or total deficiency of gastric secretion. That these do not occur after the salivary glands are extirpated with that regularity that is necessary to justify the use of the term "*hormon*," is at least partially explained by the existence of several other sources wherefrom the secretory apparatus of the stomach may receive its stimulations; these other sources have been sufficiently considered in the preceding and in the *Biochemische Zeitschrift*, Vol. xi, p. 253.

I do not wish to be understood as asserting that an extract of the inactive submaxillary gland alone can have an effect in raising the amount and proteolytic activity of gastric juice, but only, that, if it possibly could exert such an effect, not sufficient time was allowed after the injection in Dr. Loevenhart's experiments to adequately test this point of inquiry.

If there is anything of importance that has revealed itself to us since the publication in the *Biochemische Zeitschrift*, Vol. xi, p. 238, it has come through experimental study of the occasional long latent period after injection of some salivary extracts and not after others. This has suggested the existence of chemic substances which inhibit or check gastric secretion. These substances, if they exist as definite chemical bodies, must be more abundant in resting, than in functionally active, salivary glands.

There is nothing contradictory in the idea that one and the same gland cell in one segment of the digestive tract may contain two kinds of chemical messengers for the succeeding segment of the digestive apparatus. One kind stimulates secretion in the following segment and a second kind inhibits or arrests it.

Starling ("Recent Advances in the Physiology of Digestion," p. 90) speaks only of *hormones* (from *ορμάω*, to excite, arouse or stimulate). But on reflection it must be evident that for the normal regulation of life processes, it may, under certain conditions, be equally important that any process of secretion or vascular tonus should be capable of inhibition by chemical messengers. Two such diagonally opposed chemical substances which are concerned in coagulation have been isolated from lymphocytes by Lilienfeld and Delezenne, one of which *leuconuclein* favors coagulation and a second *histon* which inhibits it. The leuconuclein corresponds to the *hormones* but the *histon* is an inhibitor. For such chemic bodies — physiologic arresters like *histon* — I would suggest the name *koliones* from the Greek *κωλύων*, to inhibit, to prevent, arrest or check.

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A critical study of the conditions under which zymase and its associated co-enzyme bring about alcoholic fermentation.

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Zymase, the enzyme of yeast discovered in 1896 has since been proved by Harden and Young to consist of two parts, (1) zymase proper, an enzyme-like body possessed of high molecular complexity, non-diffusible and thermo-labile, and (2) a readily diffusible, thermo-stabile, relatively simple, chemical complex, which, for lack of a better term, has been designated as the co-enzyme of zymase.

Harden and Young separated the bodies in question by diffusion, but owing to the paucity of their materials and the destructive effect exerted by secondary causes during the lengthy process