Physiology. — *"Concerning the Sensitivity to Poisons in Animals suffering from Avitaminosis."* By W. STORM VAN LEEUWEN and F. VERZÁR. (Communicated by Prof. R. MAGNUS).

(Communicated at the meeting of November 27, 1920).

EIJKMAN found in 1893 that fowls, fed on polished rice, develop polyneuritis, and that this disease could be prevented by an undermilled rice-diet, or by adding to the polished rice the "silverlayers" detached from it. He found, moreover, that in man an abundant diet of polished rice subserved the development of beri-beri, whereas the disease was not produced, or if already produced, was cured when the silverlayers had been added to the rice. Later on it appeared that these findings bear on a special case of a general rule. Not merely in unhusked rice, but also in all sorts of foodstuffs, constituents occur that are essential for the normal growth and the healthy condition of men and animals, even though these foodstuffs contain an adequate amount of the proper, long known nutritious element. These constituents, whose real nature is unknown as yet, are often classed together as "vitamins".

Through the latest achievements in this field, notably of American workers, our knowledge of these things has largely increased. We now know that the term "vitamin" is not applied to a single substance, but that it includes various accessory foodstuffs, the fat soluble *A* and the water soluble *B*, and perhaps a third substance *C*; we are also aware that according as various nutritious elements are wanting in the foodstuffs, the symptoms of a disease may be widely different and that these symptoms may also be very different in different animals. Many experimental data concerning the occurrence of vitamins in various foodstuffs have been brought forward. There is one thing, however, of which we must still admit great ignorance, viz. the causation of the symptoms of the disease, revealing themselves in animals that suffer from a deficiency of vitamins. Some hold that under certain conditions this deficiency brings about a predisposition to infection with certain bacteria, but this supposition does not afford complete satisfaction and certainly does not clarify every case.

The symptoms of avitaminosis that present themselves are disorders

in the innervation of the striated muscles, disorders in the innervation of the unstriated muscular tissue (paralysis of the esophagus and the gastro-intestinal canal in the fowl, among others) and also trophical disorders. These disturbances are no doubt partly of a nervous character; in animals suffering from avitaminoses, e.g. in fowls attacked by polyneuritis, distinct anomalies occur in the peripheral nerves. These anatomic anomalies, however, cannot be the decisive factor in the origin of the avitaminosis, since very often in animals, exhibiting marked symptoms of avitaminosis, an injection of the vitamins concerned may exert a highly curative effect in a very short time, so that the animal may practically be cured. This leads to the conclusion that part of the disorders occurring with avitaminosis are doubtless functional, i.e. the organs of the animal do not react on the stimuli present at that moment, but may recover, or nearly so, their normal function again through the addition of a special substance: vitamin.

It should seem then that with avitaminosis the condition frequently occurs that several striated and unstriated muscles do not indeed react, but that they may be incited to reaction through the addition of a special substance.

The question, therefore, arises: Why do these striped and smooth muscles not react?

In our judgment three possibilities must be considered, anyhow so far as the unstriated muscular tissue is concerned:

Firstly, the organs do not react, because the substance which has to stimulate the organ is not present in an adequate amount.

Secondly: the organs do not react because their sensitivity to stimulating substances, even if present in an adequate amount, is lessened.

Thirdly: the sensitivity of the organs is normal, there is sufficient quantum of stimulating substances, but specific (colloidal) substances are wanting in the body of the animals, which have to facilitate or to promote the action of the stimulating substances through the organs.

An intimation that influences on the sensitivity of unstriated muscular tissue are in operation in the symptoms of avitaminosis, is found in a report by UHLMANN [1]), who showed that in a vitamin-preparation, orypan, there is a substance which plays an influence on unstriated muscular tissue which resembles the influence of pilocarpin.

Without having taken any cognizance of UHLMANN's researches,

[1]) FR. ULHMANN. Beiträge zur Pharmakologie der Vitamine. Habilitationsschrift.

VERZÁR and BÖGEL had examined the influence of extracts, which certainly contained fat soluble *A* or water soluble *B* on various surviving organs, and had found this influence to be inappreciable. However, since owing to external circumstances, they were not in a position to examine also the "vitamin"-properties of their extracts, and since their extracts were most likely different from UHLMANN's, UHLMANN's finding is by no means disqualified by their investigation.

We believe that prior to any endeavour to better understand the action of vitamins, and to realize the significance of the observations made by UHLMANN a.o., it is necessary to decide on the three possibilities suggested above.

For this reason we narrowly considered the probability expressed in the second question by trying to ascertain whether in animals suffering from avitaminosis a lessened or anyhow altered reaction on poisons could be demonstrated. Of course, if in this way an altered reaction was found, it still remained for us to decide whether this altered reaction rests on a modification of the sensitivity of the organs (compare sub 2) or would prove to depend on the possibility suggested sub 3.

We experimented with fowls and with cats.

The fowls were fed for some weeks on polished rice. As known, these animals relish this food at first, but their appetite for it gradually diminishes and soon they most often show a disinclination to eat it; then we had recourse to "forced feeding". Their reaction on poisons was not examined in these experiments until marked symptoms of polyneuritus made themselves evident; some animals were already moribund during the experiment. We anaesthetized the animals with ether, registered the bloodpressure and determined the sensitivity to adrenalin, to cholin and to histamin intravenously; we also ascertained how strong the electric current had to be for the vagus-stimulation to yield a distinct lowering of the bloodpressure, and subsequently we endeavoured to determine the quantity of atropin that was required to abolish this influence of the vagus on the bloodpressure.

After this bloodpressure-experiment the animal was killed and the gut, in some cases also the esophagus, was removed, put into Tyrode-solution and the same day or the next we determined the sensitivity of the surviving gut to pilocarpin, to atropin, afterwards also to cholin and to histamin.

Not knowing the sensitivity of normal fowls to the above-mentioned poisons we first examined four normal fowls.

In the four cats which were examined, an avitaminosis was elicited by means of a prolonged meat-diet, the meat being prepared in the manner described by VOEGTLIN [1]). The meat deprived of its fat and made alkaline, was heated to 120° in the autoclave for three hours. This meat, when neutralised by the addition of acid, was relished by the cats.

In our experiments with cats a special inquiry into the reaction of normal animals was not necessary, because we had sufficient data, already obtained in our laboratory, at our disposal.

We wish to call attention to the fact that, although the symptoms, exhibited in our animals, depended for the major part, anyhow in fowls, on a deficiency of water soluble *B*, the food was devoid not only of one but of several vitamins, and there was also a deficiency of other foodstuffs; but this did not matter in our investigation, considering that we only wished first to ascertain whether a deficiency of vitamins would at all result in a difference in sensitivity. Had this inquiry yielded positive results, we still should have had to find the special vitamin, which was the determinant factor here. Seeing that the result was negative a more detailed investigation was no longer needed.

The results of our research will be published in extenso elsewhere. Suffice it to say here that — beyond expectation — in morbid animals the reaction did not in any respect differ from that found in healthy animals. True, there occur rather marked individual deviations in sensitivity to the poisons examined, but these were not greater in the diseased animals than in the normal ones.

When we assume that many of the automatic functions of the unstriated muscles are brought about by chemical stimuli, and when we see moreover that in many unstriated muscles that function has lost much of its activity in animals suffering from avitaminosis, then the result of our researches compels us to believe that in these diseased animals there is presumably a deficiency of stimulating substances, and that the receptive organ is *not* the seat of the disturbance, and also that the decrease in activity is not brought about by a deficiency of (colloidal) substances that promote the action of poisons.

We have already pointed out that UHLMANN has established that a vitamin-preparation (orypan), examined by him, acted pharmacologically in a similar way to pilocarpin. On this finding is based

[1]) CARL VOEGTLIN and G. C. LAKE. Experimental Mammalian polineuritis produced by a deficient diet.

the hypothesis that in the case of avitaminosis an impaired function of many organs is caused by a deficiency of a substance, which is supposed to be a constituent of "orypan". We believe that this problem is not yet ripe for solution for the simple reason that only few positive facts are known. We only wish to point out that the above hypothesis migth be supported by the results of our research.

CONCLUSIONS.

When avitaminosis has been elicited in fowls through a polished rice diet, or in cats fed on specially prepared meat, the sensitivity of the animals to adrenalin, histamin, cholin, and atropin, and the sensitivity of the surviving organs of those animals to histamin, pilocarpin, atropin and cholin, is unmodified.

In two experiments it was proved that atropin (in doses of from 0,001 mgr. to 1 mgr. added to 75 c.c. of Tyrode) had no inhibitive effect on the guts of fowls suffering from avitaminosis; these guts performed only faint spontaneous movements. The gut of normal fowls displayed unmistakable inhibition on the application of atropin. In view of LE HEUX's experience of the influence of cholin on the inhibitory or the stimulating effect of atropin on the gut, this would also lend support to the conception that in hens fed on polished rice a stimulating substance in the gut is wanting.

Physics. — "*The rectilinear diameter of hydrogen*". By E. MATHIAS, C. A. CROMMELIN and H. KAMERLINGH ONNES. Communication N°. 154*b* from the Physical Laboratory at Leiden. (Communicated by Prof. H. KAMERLINGH ONNES).

(Communicated at the meeting of January 29, 1921).

§ 1. *Introduction.* This communication forms the continuation of a series of contributions to the knowledge of the density-curves for liquid and saturated vapour and of the diameters, in the case of substances of low critical temperature and simple molecular structure. The investigation by means of the dilatometer-method was started in the Leiden physical laboratory a considerable time ago and has dealt successively with oxygen [1]), argon [2]) and nitrogen [3]). Great importance was attached to the extension of the measurements to hydrogen for the knowledge of its equation of state, especially in connection with previous determinations of the liquid densities between boiling point and melting point [4]), of the critical point [5]) and the various computations of the critical density [5]) [6]).

The research could not be carried out, however, until the experimental difficulties had been overcome as regards the construction of a transparent bath of constant and uniform temperature between the critical point and the boiling point, i.e. between about — 240° C. and — 253° C.

§ 2. The *apparatus* for the compression of hydrogen, the measurement of the liquid and vapour volumes, the determination of the vapour pressures and that of the volume of the gas under

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[1]) E. MATHIAS and H. KAMERLINGH ONNES, these Proceedings 13, p. 939, Leiden Comm. N°. 117.

[2]) E. MATHIAS, H. KAMERLINGH ONNES and C. A. CROMMELIN, these Proceedings 15, p. 667, Leiden Comm. N°. 131*a*.

[3]) E. MATHIAS, H. KAMERLINGH ONNES and C. A. CROMMELIN, these Proceedings 17, p. 953, Leiden Comm. N°. 145*c*.

[4]) H. KAMERLINGH ONNES and C. A. CROMMELIN, these Proceedings, 16, p. 245, Leiden Comm. N°. 137*a*.

[5]) H. KAMERLINGH ONNES, C. A. CROMMELIN and P. G. CATH, these Proceedings 20, p. 178, Leiden Comm. N°. 151*c*.

[6]) J. J. VAN LAAR, Chem. Weekblad 16 (1919), p. 1557.