with *V. H.* an increase of more than 34%
„ *N.* „ „ „ „ „ 19%

We have previously remarked, that the increased respiratory exchange at a higher temperature cannot be attributed to this, seeing that the determination had not been made, until an equilibrium had presumably been established between internal and external gas-exchange. Indeed, the O_2-consumption and the CO_2-elimination increased more considerably than the tidal air.

Our experimental evidence seems to show that muscular work at a high temperature is less economical than at a low temperature, and also that this difference is more marked with one subject than with another.

The increase of gas-exchange parallel to the rise of temperature was not gradual, but sudden at 21°—22°.

Physiology. — *"The influence of the reaction upon the action of ptyalin"*. By Dr. W. E. RINGER and H. v. TRIGT.

(Communicated by Prof. C. A. PEKELHARING in the meeting of November 30, 1912).

One of us (v. TR.) has for some time been studying the effect of diet on the action of the diastatic enzyme of the saliva, to which the name ptyalin has been applied. The results of other researchers into this subject are to some extent conflicting with each other[1]). Nor do VAN TRIGT's experiments positively demonstrate an influence of diet. Though, taking one with another, they seemed to point to an influence, occasionally there appeared striking deviations without our being able to fix upon the cause, so that we did not know what to make of the results.

This experimentation was conducted as follows: saliva was added to amylum solutions and after some time the reducing power of the solutions was determined. This method involves the risk of fluctuations in the reaction of the fluids, e.g. such as are brought about by the flask-wall or by carbon dioxide from the air, since in approximately neutral fluids without regulating-mixtures the reaction may be considerably shifted by a trifling disturbance. This would account for the striking deviations mentioned just now, recent researches having shown that slight modifications of the reaction markedly affect the activity of enzymes.

Now if, in prosecuting our experiments, due care being taken all

[1]) Cf. HAMMARSTEN's Lehrbuch der physiologischen Chemie.

the time to obviate any noxious influence of the flask-wall or of the carbon-dioxide upon the reaction, we should detect unmistakable influence of the diet, this might be owing to various causative factors. First of all the concentration of the enzyme might have been altered by the diet. In the second place the organism might efficiently alter the concentrations of the ions, which are so material to the action of the enzyme, especially the H- and OH-ions, as well as the Cl-ions and others.

We thought proper, therefore, to cautiously watch the influence of the H- and OH-ions in order to ascertain by subsequent experiments. whether variations in the activity of the enzyme are to be attributed to changes in the concentrations of the said ions. Moreover, an accurate knowledge of the influence of these ions may lead to a clearer insight into the action of the enzyme.

Previous inquiries into the effect of acids and alkalis on the action of ptyalin yielded rather contradictory results [1]), from which it was supposed that either acids or alkalis acted favourably.

As a rule we used in our investigations the methods employed by SÖRENSEN [2]) in his remarkable experiments on enzymic actions. We adopted the following course:

filtered saliva, designated "enzyme" in the following tables, was made to act at 37° upon 1% amylum solutions. After the action of the enzyme had been arrested by heating it was estimated by the determination of the reducing power of the digestion-fluid, of the rotatory power and by reaction with iodine. Various reactions were given to amylum solutions. To obtain them and to maintain them constant three buffing- or regulating-mixtures were applied, viz.

1. phosphate-mixtures,
2. citrate-mixtures,
3. acetate-mixtures.

The process of digesting lasted 20 minutes for all series of experiments but one.

1. *Experiments with phosphate mixtures.*

(all the glass vessels had been exposed to steam for 15 minutes.)

Into ERLENMEIJER-flasks (Jena-glass), capable of holding 300 c.c. were placed:

10 c.c. of a phosphoric acid solution 1.485 n., varying amounts of sodium hydrate 0,5670 n., and water up to 50 c.c. To this 200

[1]) Cf. HAMMARSTEN'S Lehrbuch der physiologischen Chemie.

[2]) Comptes rendus des travaux du Laboratoire de Carlsberg. 8me Vol. 1r. Livraison 1909.

c.c. of the amylum solution was added by means of a pipette. As a matter of course, all the tests of the same series were made with the same freshly prepared solution, which was obtained by mixing 25 gr. of dried amylum with one liter of water and heating it to the boiling point, while stirring the fluid and maintaining this temperature for about a minute. After cooling the mixture was made up to 2 liters [1]) and filtered through glass-wool or muslin.

The flasks holding the phosphate-mixtures and the amylum, were first heated to 37° and then maintained at this temperature in the thermostat for at least 20 minutes previous to the addition of the enzyme. After the enzyme had been working on for 20 minutes, the flask was dipped into a boiling waterbath and was constantly and regularly moved, always in the same manner, till a temperature of 90° was reached, so that every time the action of the enzyme was arrested in the same way.

The reducing power of the cooled fluid was determined after BERTRAND and was expressed in m. Gr. copper per 100 c.c. of the fluid.

The determination of the reaction was performed electrometrically. The hydrogen-electrodes were treated after HASSELBACH'S [2]) shaking method, and measured by means of mercury-calomel-electrodes with normal and $^1/_{10}$ n potassium chloride The reaction is expressed in p_H: the negative logarithm of the hydrogen-ions-concentration.

The following tables show the results of the most important series of experiments.

1st Series of experiments. Enzyme v. *T.*

Nr.	Phosphoric acid solution c.c.	NaOH c.c.	H_2O c.c.	Amylum c.c.	Enzyme c.c.	Reduction m.Gr. Cu	Rotation minutes	Iodine reaction	p_H
1	10	13.4	26.6	200	2	71.10	—	—	5.186
2	10	13.7	26.3	200	2	182.15	—	—	5.69
3	10	14	26	200	2	212.30	—	—	5.80
4	10	15	25	200	2	218.95	—	—	6.22
5	10	16	24	200	2	214.85	—	—	6.40
6	10	18	22	200	2	176.50	—	—	6.78

[1]) Occasionally 4 liters had to be made.

[2]) Biochemische Zeitschrift, Bd. 30, p. 317.

2d Series of experiments. Enzyme *R* diluted with 3 vol. of water.

Nr.	Phosphoric acid solution c.c.	NaOH c.c.	H_2O c.c.	Amylum c.c.	Enzyme c.c.	Reduction m.Gr. Cu	Rotation minutes	Iodine reaction	p_H
1	10	13	27	200	2	Reduction not perceptible	±192	blue	4.53
2	10	13.5	26.5	200	2	180.10	188	blue, shade of violet	5.33
3	10	14	26	200	2	234.80	186	violet, shade of blue	5.86
4	10	14.5	25.5	200	2	**244.55**	185	violet	**6.05**
5	10	15	25	200	2	235.'0	186.5	violet, shade of blue	6.24
6	10	15.5	24.5	200	2	223.60	188	violet-blue	6.30
7	10	17	23	200	2	179.10	191.6	blue, shade of violet	6.61
8	10	20	20	200	2	105.40	195	blue	7.01

3d Series of experiments. Enzyme *D.*

Nr.	Phosphoric acid solution c.c.	NaOH c.c.	H_2O c.c.	Amylum c.c.	Enzyme c.c.	Reduction m.Gr. Cu	Rotation minutes	Iodine reaction	p_H
1	10	13.2	26.8	200	2	106.45	194	blue	4.90
2	10	13.5	26.5	200	2	194.50	190.3	blue, shade of violet	5.52
3	10	14	26	200	2	251.25	190	violet-blue	5.83
4	10	14.5	25.5	200	2	270.10	189.7	violet, shade of blue	6.08
5	10	15	25	200	2	**271.20**	188	violet	**6.19**
6	10	15.5	24.5	200	2	265.55	191	violet, shade of blue	6.37
7	10	17	23	200	2	220.55	192	violet-blue	6.61
8	10	20	20	200	2	156.60	195	blue	7.03

From these experiments it appears, that the concentration of the hydrogen-ions exerts a considerable influence upon the action of the enzyme; further that an increase of c_H, consequently a decrease of p_H accelerates its activity. until a certain optimum is reached, after which the action slackens again. We also observe the same behaviour with enzymes from different sources, however with a noticeable difference in their activity. From another series of experiments we gathered that the optimal reaction lies at about the same point in much more dilute phosphate solutions; we also learnt, that all over the series the action of the enzyme was more vivid. It follows

then, that phosphate-mixtures are inhibitive to the action; less so in highly dilute than in the concentrated solutions.

2. *Experiments with citrate-mixtures.* A citrate solution was made from 275 gr. of pure citric acid (pro analysi), 105 gr. of NaOH (MERCK's e natrio pro analysi) and water to 1 liter. 20 c.c. of this citrate solution diluted with water to 250 c.c. yielded $p_H = 4.915$.

4th Series of experiments. Enzyme *R*.

Nr.	Citrate solution c.c.	NaOH c.c.	H_2O c.c.	Amylum c.c.	Enzyme c.c.	Reduction m Gr. Cu	Rotation minutes	Iodine reaction	p_H
1	10	14.7	25.3	200	2	247.60	195	bluish-violet	5.99
2	10	19.57	20.43	200	2	357.15	189	reddish-violet	6.49
3	10	19.94	20.06	200	2	380.15	189	red, shade of violet	6.526
4	10	20.40	19.6	200	2	380.65	188	reddish-brown	6.62
5	10	21.3	18.7	200	2	**396.00**	187	reddish-brown	**6.73**
6	10	22.1	17.9	200	2	358.65	187	red, shade of violet	7.09
7	10	23	17.0	200	2	183.15	197	blue, shade of violet	7.425

5th Series of experiments. Enzyme *R* diluted with 1 vol. of water.

Nr.	Citrate solution c.c.	NaOH c.c.	H_2O c.c.	Amylum c.c.	Enzyme c.c.	Reduction m.Gr. Cu	Rotation minutes	Iodine reaction	p_H
1	5	5.0	40	200	2	81.35	202.7	blue	5.80
2	5	8.20	36.8	200	2	139.70	200	blue	6.26
3	5	9.78	35.22	200	2	**158.10**	197	blue, shade of violet	**6.55**
4	5	10.20	34.80	200	2	147.85	199.3	blue, shade of violet	6.74
5	5	10.65	34.35	200	2	128.45	201	blue	6.85
6	5	10.90	34.10	200	2	107.95	202.7	blue	7.046
7	5	11.05	33.95	200	2	90.05	204	blue	7.11
8	5	11.30	33.70	200	2	60.90	204.5	blue	7.41
9	5	11.60	33.40	200	2	reduction not perceptible	205	blue	7.497

Here again an optimal reaction is educed, which, however, has slightly shifted towards the neutral point. A decrease of concentration diminishes this deviation.

3. *Experiments with acetate-mixtures.* A solution of sodium acetate (170 gr. per liter) was mixed with different quantities of 1 % acetic acid. The following experiments were made:

6th Series of experiments. Enzyme R. diluted with 3 vol. of water

Nr.	Acetate solution c.c.	Acetic acid solution c.c.	H_2O c.c.	Amylum c.c.	Enzyme c.c.	Reduction m.Gr. Cu	Rotation minutes	Iodine reaction	p_H
1	20	0	30	200	2	47.60	turbid	blue	7.297
2	20	1	29	200	2	137.65	202	blue, shade of violet	6.65
3	20	2	28	200	2	182.65	199	bluish-violet	6.55
4	20	4	26	200	2	221.05	198	bluish-violet	6.21
5	20	5.6	24.4	200	2	**222.05**	195	violet-blue	**6.106**
6	20	7	23	200	2	221.55	197	violet-blue	5.98
7	20	12	18	200	2	200.05	199	bluish-violet	5.78
8	20	30	0	200	2	118.20	200	blue	5.37

Again an optimal reaction is evolved; it is equal to that of the phosphate solutions. On either side of it the action of the enzyme diminishes, first slowly, then rapidly. The optimal reaction lies in phosphate solutions at $p_H = 6.05$, as may be seen from a graphic representation of the reduction as function of the p_H. In acetate solutions we find $p_H = 6.08$, whereas in citrate-experiments values vary according to the concentration. In the 5th series we found an optimal reaction $p_H = 654$.

All values of p_H communicated thus far, were estimated at 18°. They are somewhat different at 37°, the temperature at which the experiments were made. The reactions of the fluids, that were optimal, have also been determined by us. We found:

in the phosphate solutions $p_H = 6.00$

in the citrate solutions (10 c. c. of citrate 4th series) $p_H = 6.86$

in the acetate solutions $p_H = 6.028$.

The neutral point lies at 37° at $p_H = 6.796$.

For purposes of comparing the action of the various regulating-mixtures we carried out the following experiment. (p. 805).

It is evident from this test that, the reaction being neutral, the influence of phosphate is inhibitory; when the reaction is slightly acid ($p_H = 6.5$; a neutral reaction is not easily obtained with citrate)

7th Series of experiments. Enzyme R diluted with 1 vol. of water.

	Regulating mixture	H_2O c.c.	Amylum c.c.	Enzyme c.c.	Reduction m.Gr. Cu.	Reaction (determined at 18°) p_H
a	none	50	200	2	318.20	electrometrical determination not practicable on account of the lack of electrolytes. Neutral behaviour to litmus, so $p_H \pm 7.07$
b	10 c.c. phosphoric acid 20.6 c.c. NaOH	19.4	200	2	245.05	7.07
c	10 c.c. phosphoric acid 16.25 c.c. NaOH	23.75	200	2	425.15	6.50
d	10 c.c. citrate, 19.55 c.c. NaOH	20.45	200	2	221.55	6.468

a comparison between citrate and phosphate shows that inhibition is much stronger with the former than with the latter.

From the removal of the optimal reaction towards the neutral point, as well as from the tests published in this paper, it is apparent, that citrate inhibits most strongly on the side of the minor p_H's, and that this impeding action weakens towards the neutral point.

The optimal reactions being identical in phosphate- and acetate-mixtures, it was likely, that either of them should slacken the action of the ptyalin in the same way. The following test illustrates the fact that, if the reactions are the same, both mixtures equally affect the enzymic action.

8th Series of experiments. Enzyme *R* diluted with one vol. water.

	Regulator	H_2O c.c.	Amylum c.c.	Enzyme c.c.	Reduction mGr. Cu	p_H
a	10 c.c. of acetate 5 c.c. of acetic acid	35	200	2	489.2	5.886
b	10 c.c. of phosphoric acid 14 c.c. NaOH	26	200	2	483.5	5.886

We now passed on to inquire how this influence of the reaction upon the action of ptyalin is to be accounted for. It may indeed be imagined, that H-ions favour the enzymic action, but how is it then that beyond the optimal c_H they largely impede the activity. Is it perhaps to be attributed to an injury to the enzyme? In order to find this out we made the following experiments:

9th *Series of experiments.*

a. 10 c. c. of phosphoric acid, 13 c. c. of sodium hydrate and 27 c. c. of water were mixed at room-temperature with a mixture of 25 c. c. of enzyme R + 25 c. c. of water. We examined directly the activity of this mixture, in which the enzyme had been diluted four times. It was subsequently warmed to and maintained at 37°, while at various intervals the action was noted, every time by allowing 2 c. c. to act upon mixtures of phosphate and amylum of the optimal reaction.

Nr.	Time (minutes) during which the enzyme-mixture was maintained const. at 37°	Reduction m.Gr. Cu.	Rotation minutes	p_H (if determined).
1	0	177.55	194.3	6.06
2	8.75	179.10	—	—
3	16.75	179.10	193.0	6.00
4	41.75	179.10	—	—
5	88.75	179.10	193.0	6.075
6	178.75	181.60	—	—
7	268.75	179.10	193.0	5.975

The p_H of the enzyme-mixture was 5.502.

b. 10 c. c. of phosphoric acid, 12 c. c. of sodium hydrate, 28 c. c. of water. Addition: 25 c. c. of enzyme R + 25 c. c. of water, amylum solutions as in the preceding test; p_H of the enzyme-mixture 4.095.

Nr.	Time (minutes) during which the enzyme-mixture was maintained const. at 37°	Reduction m.gr. Cu.	Rotation minutes	p_H
1	0	155.00	201.0	5.98
2	18	147.85	201.7	6.04
3	47.5	139.70	199.0	6.02
	fresh enzyme-mixture made of the same Enzyme *R* and the same p_H.			
4	0	162.25	199.3	6.03
5	138	113.10	201.5	6.08
6	373	56.30	203.0	6.03

Our results show that the enzyme is not yet injured at $p_H = 5.5$, but is gradually injured at $p_H = 4.095$. However, in view of the relatively short duration (20 min.) of the digestion-experiments described above, the injury is, even in the case of $p_H = 4.095$ only of small account. We conclude, therefore, that the inhibitory influence of the H-ions in concentrations beyond the optimal is not attributable to injury to the enzyme.

In addition we have also tried to ascertain, whether the enzymic activity is weakened in fluids made slightly alkaline.

c 10 c.c. of phosphoric acid, 27 c.c. of sodium hydrate, 13 c.c. of water. Addition: 25 c.c. of enzyme R + 15 c.c. of water, all the amylum solutions as in the preceding test, p_H of the enzyme-mixture, 8.718.

Nr.	Time (minutes) during which the enzyme-mixture was maintained const. at 37°	Reduction m.Gr. Cu.	Rotation minutes	p_H
1	0	142.20	—	—
2	29.5	147.35	—	6.02
3	55.5	147.35	—	—
4	103.5	147.35	—	—
5	255.0	140.70	—	—
6	380.5	134.55	—	—

Consequently no injury in two hours' time with a faintly alkaline reaction, $p_H = 8.718$.

It is obvious, therefore, that in our experiments injury to the enzyme cannot have had any influence worth mentioning; on this account we could not expect the optimal reaction to shift in a prolonged digestion-test. Researches, each lasting 100 minutes, 5 times longer than the other experiments, confirmed our supposition.

Further experimentation will have to reveal the relation between the electric charge of ptyalin to its action, for which the iso-electric point has to be determined [1]).

Summary.

For the action of ptyalin the concentration of the hydrogen-ions is highly important. In fluids in which the reaction has been deter-

[1]) Cf. MICHAELIS Bioch. Zeitschr. Bd. 35, S. 386, Bd. 36. S. 280.

mined by phosphate- and acetate-mixtures, we found at $p_H = 6.00$ an optimal reaction to the action of the enzyme. On either side the action decreases, first slowly, afterwards rapidly. Even at $p_H = 4.5$ and 7.5 it is stopped almost completely. At these p_H's injury to the enzyme is out of the question during the whole time of the test. The place of the optimal p_H does not change even when the digestion-time is five times the ordinary duration. The influence of citrate-mixtures is much more inhibitory than that of phosphate- and acetate-mixtures. The inhibition is energetic especially on the side of the minor p_H's. This accounts for the fact that in citrate-mixtures the optimal reaction has shifted towards the neutral point.

Astronomy. — *"On absorption of gravitation and the moon's longitude."* By Prof. Dr. W. DE SITTER. Part I.

(Communicated in the meeting of November 30, 1912).

By absorption of gravitation we mean the hypothesis that the mutual gravitational attraction of two bodies is diminished when a third body is traversed by the line joining the first two. If this absorption exists, it will manifest itself by diminishing the attraction of the sun upon the moon during a lunar eclipse. Therefore, in order to test the reality of our hypothesis, we must compute the perturbations in the longitude of the moon which are a consequence of this decrease of attraction, and compare these computed perturbations with the well known deviations of the observed longitude from that derived in accordance with the rigorous law of NEWTON. NEWCOMB, in the last paper from his hand (M. N. Jan. 1909) has put before the scientific world the great problem of these deviations or "fluctuations" in the moon's longitude. They can be represented by a term of long period, for which NEWCOMB finds an amplitude of 12".95 and a period of 275 years (great fluctuation), upon which are superposed irregular deviations (minor fluctuations), which amount to not more than $\pm$ 4" in NEWCOMB's representation. Mr. F. E. Ross, NEWCOMB's assistant, has afterwards represented these minor fluctuations by two empirical terms having periods of 57 and 23 years and amplitudes of 2".9 and 0".8 respectively (M. N. Nov. 1911). The outstanding residuals are very small: after 1850 they seldom reach 1". In the years before 1850 the minor fluctuations are not so well marked, probably because (owing to the smaller number and greater uncertainty of the available observations) too many years have been combined in each mean result.