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in (14) the curve makes a much more acute bent, descends with far greater rapidity to the minimum and ascends far more rapidly too, than the curve that may be deduced from their experiments.

Whilst the possibility is not excluded that by our law the relation between magnitude of stimulus and effect is rendered with some accuracy for the sense of weight, such is not the case for the senses of sight and hearing, within the limits of observation at least.

I trust soon to be able to show, that this fact may be the consequence of a biological correction, quite independent from accommodation or pupil-alteration, caused solely by addition of stimuli, and accompanying every stimulation of sense.

Bacteriology. — "*The Physiological Bacteriology of the intestinal canal (2nd abridged paper: The Bacteriological relations in the intestinal canal of the rabbit).*" By Dr. ALEX KLEIN. (Communicated by Prof. PLACE).

Already PASTEUR fixed the attention on the great signification of the question in how far the numerous lower organisms, present in the intestinal canal of man and animals, play a part in the digestion. Since that time (1885) an extensive literature on the bacteriology of the intestinal canal has appeared; the results of these researches are in brief as follows:

In the first part of the small intestine of most animals, if no ingesta are present, no, or at least very few bacteria are found, (auto-sterilisation of KOHLBRUGGE); downward in the small intestine the number increases (NENCKI, GILBERT-DOMINICI, BROTZU, ESCHERICH i. a.). A very considerable increase is observed in the Coecum (ALAPY, ESCHERICH, KOHLBRUGGE i. a.), whilst in the rest of the large intestine now an increase, then a diminution of the number of lower organisms may be stated. Basing on these observations and at the same time in consequence of direct experiments conducted in that direction (BIENSTOCK, VINCENZI, SCHÜTZ), the existence of an anti-bacterial action in the small intestine is admitted. This anti-bacterial action, by killing the "wild germs", introduced together with the food, is cause of the restriction of the putrefaction processes in the intestinal canal. In the Coecum there is no such an anti-bacterial action; it has a flora of its own, consisting of the "obligative intestinal bacteria" (chiefly B. coli and allied organisms), of which a symbiose with the mucous membrane of this portion of the intestinal canal must be accepted (SCHOTTELIUS, KOHLBRUGGE); this flora is of

importance for the digestion. Moreover, in consequence of the results obtained by painstaking and interesting experiments on animals, bred sterile from their birth, the influence of the intestinal bacteria on digestion, has been denied by some (NUTTALL and THIERFELDER), confirmed by later researches (SCHOTTELIUS).

The foundation of these numerous investigations, the determination of the number of bacteria in the different parts of the intestinal canal, must be called an erroneous one, in as much as all these experiments relate only to the living bacteria present in the intestinal canal. And as little as the sanitary condition of a population can be judged only after the number of living individuals present at a given moment, or the murderousness of a battle exclusively after the number of remaining soldiers, without taking into consideration the killed, as little is a right insight to be acquired into the sanitary condition of the bacterial population of the intestinal canal and the battles fought there between these lower beings and the living animal organism (anti-bacterial influences), by only looking at the living individuals, leaving the dead ones out of account.

The proportion existing between the number of living and of dead individuals I call the *sterility-index*; this proportion thus indicates the degree of sterility reached by a determined population of bacteria.

The sterility-index of a bacterial population is estimated from the relation of two data: 1st The difference in number of this population found between the culture-method and the microscopic counting-method (proportional number), and, 2nd the determination of that fraction of the microscopically counted organisms, which are still able to propagate.

The latter determination is effected *biologically*: the living organisms in the bacterial population are allowed to propagate and after a certain time the number is again determined, as well microscopically as by culture. In order to accomplish this propagation the same medium is by preference used in which the original bacterial population developed, as the lower organisms also have in the beginning been able to increase in this medium. Only it will be necessary by dilution sufficiently to remove an eventually active anti-bacterial action, which originates from the fluids of the human or animal body. The moment for the second determination should in each special case be fixed experimentally. The difference in time must not be too short, else the increase of living organisms is too slight to produce a distinct augmentation of the number of microscopically counted

bacteria; and, again, the quickness of increase of the living individuals corresponds with the nature of the food, the presence of anti-bacterial influences, etc. Nor must this period be taken too long because finally the dead individuals decompose and vanish.

If the excess of microscopically counted bacteria prove to have completely died, the sterility-index is directly found by diminishing the original proportional number with 1; this is for instance the case in human faeces [1]). But when only a fraction died, so that part of the microscopically counted organisms are alive but do not develop on our usual culture-media, then, for the estimation of the sterility-index from the data found, it is admitted that the living but not cultivable individuals have multiplied during the period of observation in the same measure as the cultivable ones.

The determination of the sterility-index might also directly be made *microscopically*, if the bacteria that have died already before, could be distinguished by microscopically perceptible changes from those organisms which in the beginning were alive. Indeed, after death there occur modifications in the bacterial bodies, at least in the intestinal canal of the rabbit, which considerably alter the pigment-absorbing faculty of these organisms. In general three stadia of decomposition may be distinguished:

1st. The *granule-stadium*, where one or two (seldom more) very darkly coloured granules may be observed in a for the rest lightly coloured stroma of the bacterial body;

2nd. The *shade-stadium*, the dark granules are no more present and the bacterial stroma is still lighter coloured; and

3rd. The *membrane-stadium* in which the bacterial stroma absorbs no pigment at all; at least there remains nothing but a fine coloured line, which still very markedly and sharply indicates the margin of the original organism.

The greater part of these decomposing bacteria are so fragile that they can but be observed by the use of the delicate treatment of the „moist staining"; when applying KOCH's staining-method they mostly fall asunder at the drying and flaming of the preparations and then form what has been by some described as bacterial detritus.

These post-mortem bacterial phenomena cannot however at first be used for the determination of the index of sterility, as they do not appear directly, but only some time after the death of the

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[1]) See my Paper „Bacteriological investigations of human faeces", Proceedings Royal Acad. of Sciences, Amsterdam. Vol. IX, p. 57.

bacteria; hence a greater number of dark and evenly coloured organisms are microscopically detected than corresponds with the number of living bacteria.

Perfectly healthy rabbits were killed by the blow on the neck; directly after death the abdomen was opened, the bowels were bound off with sterilised silk threads and the contents of the different parts of the intestine were introduced into sterilised mortars under aseptic precautions. By means of sterilised pestles the substance was evenly mixed in the mortar; when making the dilutions the intestinal contents were for some time thoroughly shaken in sterile flasks with porcelain balls. In particular for the contents of the small intestine which mostly consist of a viscid, sticky mass, in which the lower organisms are most unevenly distributed, such a laborious manipulation cannot be dispensed with in order to get a homogeneous emulsion. Nearly always all the contents of each portion of the intestine were used for the research, with exception only of the Coecum, of which after previous emulsion, 10 grs. at least were used.

In the different parts of the intestinal canal there now appeared to be a great disproportion between the number of cultivated and that of microscopically counted bacteria; the culture under other circumstances or on other nutrient media had no perceptible influence on the surplus of microscopically countable bacteria.

TABLE I.

Parts of the intestine of rabbit n° 2.	Times of the research.	Number of bact. found by micr. counting in 1 mgr of contents.	Increase with	Numbers of bacteria found by culture in 1 mgr. of contents.	Increase with
Small intestine....	directly	685 000	206.000	[1 organism on 5 mgrs.]	275.000
	24 hours at 37° C	891 000		275.000	
Coec. and Proc. vermiform.	directly	26 975.300	more than 12½ millions	136	more than 12½ millions
	24 hours at 37° C.	39.792.000		12.558.700	
Large intestine and Rectum	directly	8 565.000	nearly 9½ millions	67	nearly 9½ millions
	24 hours at 37° C	17.937.000		9.432.000	

When the sterility-index of the different parts of the intestinal canal is determined it appears that the great excess of microscopically countable bacteria *as a whole* consists of dead organisms ; hence, the sterility-index can be directly found by diminishing the proportional number with 1.

T A B L E II.

Rabbit n° 5.

Parts of the intestine.	Contents (in Grams).	Solid subst. in pCt.	Total numbers.		In 1 mgr. of contents		Sterility-index.	
			Microscop counted.	Cultiv.	Microscop.	Cultiv.		
Small intestine (upper portion)	18	7.89	9.550 967.000	[5 940]	530.600	[about 1 on 3 mgrs.]	1.607.905	169.942
Id. (downmost port. 1 M.)	3 5	8.49	2 870.175.000	67.150	820.000	19	42 741	
Coec, Pr. vermif. and Col adsc.	111	15.61	7 555 215 000 000	7 770.000	68.065 000	70	972.356	
Large intestine and Rectum .	10	34.26	176.070 000 000	255 800	17.607.000	25	688 310	

When considering the column „cultivated" organisms, quite the same relations are met with as described till now: very few bacteria in the first portion of the small intestine; the number increases in the end of it, but a vigorous augmentation appears in the Coecum, Processus vermiformis, and Colon adschendens, whilst in the rest of large intestine and Rectum again a decrease may be observed.

Quite otherwise, however, the image becomes when viewing at the last column, representing the sterility-indices of the different portions of the intestine: whilst the sterility-index of the whole small intestine is 169942, that index mounts in the Coecum to 972356; the increase in number of living organisms in the Coecum is accordingly only an apparent one; on the contrary, instead of an increase we find in the Coecum, with a more than five times greater index of sterility, that no less than 80 pCt. of the original number of bacteria which arrived living in the Coecum are dead.

In the rest of the large intestine and in the Rectum the sterility-index (688310) has become a little lower than in the Coecum. It may become lower by an increase of the number of living individuals, but also by the decrease of dead organisms; the latter being the case here. The proportion of solid substance in large intestine and Rectum has mounted to 34.26 pCt., thus more than two times that of the Coecum, hence, we might be expect here per mgr. at least twice as many microscopically countable organisms. Instead of 2×68.065000, however, only 17.607000, are found, which consequently proves that a large number of dead bacteria are decomposed and have disappeared, yet a considerable number of bacteria that had remained in the Coecum, have died, as this number should at least have mounted to 2×70 per mgr. whilst only 25 per mgr. were found.

In the whole small intestine no ingesta were present. The number of microscopically counted bacteria in the hindmost part of the small intestine, is in accordance with the higher rate of solid substance somewhat greater per mgr. than in the superior part; still the sterility-index of the former is much lower than in the latter. Hence, in that first portion there must have been a considerable dying of bacteria, whilst, as a matter of course, the 2nd part has only for a shorter time been free from ingesta and accordingly also contains a greater number of living organisms. After the passing of the ingesta the living organisms thus die off largely in the small intestine, so that the number of living individuals there may finally grow very small; and at the irregular distribution of the bacteria in the viscid-mucous substance, there may then be found relatively large sterile quan-

TABLE III.

Rabbit n° 8.

Parts of the intestine.	Contents [in grams].	Solid substance in pCt	Total numbers. Microscopically counted.	Total numbers. Cultiv.	In 1 mgr. of contents. Counted.	In 1 mgr. of contents. Cultiv	Sterility-index.	
Duodenum and Jejunum	6 250	6.07	4 969 593.750	1 581	795 135	1 organism on 4 mgrs.	3.140.791	2 586.626
Ileum	11 500	8.37	13.517 031.000	5 566	1.175.394	1 organism on 2 mgrs	2.428 499	
Coecum, Pr. vermif and Col adsc	105.500	13 84	5 977 793.525.000	632 472	56.661 550	6	9.451 466	
Large intestine and Rectum	5.500	28.33	190 329 521 250	84.095	34 605 367	15	2 263 266	

TABLE IV.

Rabbit n° 4.

Parts of the intestine.	Contents [in grams]	Solid substance in pCt	Total numbers Microscopically counted	Total numbers Cultiv.	In 1 mgr. of contents. Counted.	In 1 mgr. of contents. Cultiv.	Sterility-index.	
Small intestine (upper part)..	12.250 (ingesta)	13.60	8.189 500.000	1 447.674	668 500	118	5.656	8.180
id. (downmost 75 cm.)	4.750	15.31	3.696.385.000	5 058	778 000	1	730 798	
Coecum, Pr. vermif. and Col. adsc	127.200	22 73	607.316 400 000	3.816 000	4 774 000	30	159 149	
Large intestine and Rectum	7.500	40.23	50.599 000.000	468.000	6.746.000	62	108.116	

tities (e. g. loops of some mgrs.). Still the contents of the small intestine never prove quite sterile; an absolute sterility of the small intestine (so-called auto-sterilisation) is not attained in any case. To be convinced of this it is only necessary to use a more refined method

of examination. If all the contents of the small intestine in which there are but few living bacteria are placed at 37° C., in dilute state, in order to remove an eventually present anti-bacterial action, so that those few bacteria are allowed to propagate, then, after a

TABLE V.

Rabbit n° 3

Parts of the intestine.	Contents [in grams].	Solid substance in pCt.	Total numbers.		In 1 mgr. of contents.		Sterility-index.	
			Microscopic. counted	Cultiv.	Counted.	Cultiv.		
Small intestine (Superior part)	12	8.93	2.509.125.000	—	209.000	—	—	63.392
Small intestine (Inferior 75cms.)	4 (ingesta)	11.63	1.317.900.000	60.465	329.000	15	21.795	
Coecum, Pr. vermif. and Col. adsc.	82	24.55	5.191.109.162.000	10.297.560	63.306.209	126	504.109	
Large intestine and Rectum	6	40	34.189.921.000	1.416.000	5.698.320	236	24.144	

TABLE VI.

Rabbit n° 7.

Parts of the intestine.	Contents [in grams].	Solid substance in pCt.	Total numbers.		In 1 mgr. of contents.		Sterility-index.	
			Microscopic. counted.	Cultiv.	Counted.	Cultiv.		
Duodenum and Jejunum.....	27.5	8.17	19.965.000.000	15.125	726.000	about 1 on 2 mgrs	1.320.000	465.847
Ileum..................	39 (ingesta	11.19	48.537.060.000	131.923	1.244.540	3	368.931	
Coecum, Pr. vermif. and Col. adsc.	109.3	22.70	895.524.135.000	1.789.360	8.193.267	16	499.893	
Large intestine and Rectum	25.2	35.71	140.260.428.000	263.340	5.565.890	10	532.620	

certain number of hours, a great many organisms are found. Whilst at first in the superior part of the small intestine on each quantity of 3 mgrs. only one living bacterium was to be found, the same quantity, placed in a dilution of 1 : 11 at 37° C., after 21 hours, and estim-

TABLE VII.

Rabbit n° 6.

Parts of the intestine.	Contents (in grams).	Solid substance in pCt.	Total numbers.		In 1 mgr. of contents.		Sterility-index.
			Microscopic. counted.	Cultiv.	Counted.	Cultiv.	
Duodenum and Jejunum.....	12.5	9.53	24.199.725.000	—	1 936 000	not 1 on 6 mgrs.	—
Ileum.....	30 (ingesta)	12.96	280.021.500.000	137.499	9.334.000	4	2.036.534 / 2.2120533
Coecum, Pr. vermif. and Col. adsc.	81	22.03	6.098.909.256.000	1.113.750	75.295 176	13	5.476.011
Large intestine and Rectum.	9.5	39.45	124.577.055.000	136.875	13.133.374	14	910.151

ated for 1 mgr. of the original contents, showed by means of culture-method 2.660.000 living organisms. If the small number of living bacteria present in the small intestine in absence of ingesta, is taken into consideration it is commonly possible, either by plate culture or by different dilutions in bouillon, to determine the number of these living bacteria.

In rabbit nº. 8 (Tab. III), without ingesta in the small intestine, quite the same relations are observed. Probably the Ileum here already for a long time contained no ingesta; hence the sterility-index is higher than in the former case. The Duodenum and Jejunum which have undoubtedly already for a longer time been free from ingesta possess a still higher sterility-index. In the Coecum, Processus vermiformis, and Colon adschendens the sterility-index again rises much, whilst in the rest of the large intestine and the Rectum again decomposition of dead organisms has taken place, and, in relation to the rate of solid substance, neither increase nor diminution of living bacteria has occurred. (Table IV, p. 483).

If ingesta are present in the superior portion of the small intestine, the sterility-index is of course very low; the second part of the small intestine which for a longer time already contained no ingesta, shows a very high index. In the Coecum the index is again many times higher than in the whole small intestine.

If ingesta are present in the Ileum, whilst Duodenum and Jejunum are devoid of them, (Tab. V, VI and VII), these ingesta prove still to contain a great number of living organisms; the sterility-index is then low there; in the Coecum, Proc. vermiformis, and Colon adschendens it is, however, always higher than in the whole small intestine. As the ingesta from the second part of the small intestine arrive at the Coecum with a relatively low index, the dying of living bacteria there is evidently much more considerable than might be supposed from the comparison of the sterility-index of the Coecum with that of the whole small intestine, especially if the latter contain no ingesta at all.

Accordingly the bacterial population in the intestinal canal of the rabbit has the following course. With the ingesta large numbers of living and dead bacteria come from the stomach into the small intestine. As they move on, a number of living bacteria that have remained in the parts of the small intestine devoid of ingesta, die off, without these parts, however, becoming altogether sterile. When the ingesta have entered the large intestine the same process takes place in the inferior part of the small intestine. Furthermore, in the Coecum, Processus vermiformis, and Colon adschendens there

is a dying on great scale of living lower organisms. In the rest of the large intestine and the Rectum in not one case increase is observed, in most cases on the contrary, a continuous dying away. From the Duodenum down to the Rectum nowhere an increase of bacteria; mostly from beginning to end throughout the intestinal canal a continuous annihilation of the living bacteria. Bacteria are in the intestines of a rabbit as an army passing through the country of the enemy, being continually decimated.

In the Coecum, Processus vermiformis, and Colon adschendens the greatest mortality by far is observed. This may be nearer pointed out if, using one and the same dilution, the living organisms present in the different parts of the intestinal canal are compared. The after-action of the influences which during the life of the animal provide for the extermination of the bacteria in the intestinal canal, also proves to be most vigorous in the Coecum, Processus vermiformis, and Colon adschendens.

TABLE VIII.

No. of the rabbit.	Parts of the intestine.	Time at 37° C.	Degree of dilution.	Original number in 1 mgr. of contents.	Final number in 1 mgr. of contents.	Number of times that the original number has increased.
8.	Duodenum and Jejun	9½ hours	1 : 33	1on4mgrs	96	384
	Ileum.	id.	1 : 33	1on2mgrs	673	1.346
	Coecum, etc.	id.	1 : 33	6	137	23
	Large intest.andRect	id.	1 : 33	15	1.195	79
7.	Duod. and Jejunum.	20 hours	1 : 11	1on2mgrs	21.000	42.000
	Ileum.	id.	1 : 11	3	243.000	81.000
	Coecum, etc.	id.	1 : 11	16	108 000	6.750
	Large intest. andRect.	id.	1 : 11	10	130.000	13 000
5.	Duod. and Jejunum.	21 hours	1 : 11	1on3mgrs	2.660.000	7.980.000
	Ileum.	id.	1 : 11	19	1 543.000	81.000
	Coecum, etc.	id.	1 : 11	70	726.000	10.300
	Large intest.and Rect	id.	1 : 11	25	1.831.000	73.000

Notwithstanding in most cases more living organisms per mgr. are originally to be found in the Coecum than in the other parts

of the intestinal canal, they multiply in equal times, much less vigorously in the former than in the latter.

If thus we arrive at the conclusion that in not one locality of the intestinal canal an increase of lower organisms is seen, there can be no question of that canal having "a flora of its own", nor of a distinction between "obligative" and "facultative" intestinal bacteria. Indeed, we find in the whole intestinal canal the same species of organisms, chiefly Coli and Coliform bacteria, probably because these organisms are so universally distributed in nature and, accordingly, with the food get in large numbers into the intestinal canal. Furthermore, because by their great resistance, — which is also shown by their ubiquity in nature, — they are able longest to resist the anti-bacterial influences of the intestinal canal. At those places of the small intestine where ingesta are present, there are commonly found a number of other bacteria too. On the culture-plates these species, either by the quickness with which they liquefy the gelatin, or by the great number in which they are eventually present, may prevent the appearance of Coliform bacteria. The greater part of these organisms possess, however, a much slighter power of resistance than the bacteria of the Coli-group, and thus die first, so that in the Coecum, Processus vermiformis, and Colon adschendens chiefly part of these Coliform bacteria alone are found in living state.

Putrefaction is excluded from the intestinal canal of the rabbit, bacteria not increasing there at all.

TABLE IX.

Rabbit	Contents in grams	Total numbers in the whole intestinal canal.		Sterility-index of all the contents of the intestine.	On 1 million of dead organisms are alive.
		Microscopic. counted.	Cultiv.		
N° 1	70	945 727 500 000	14 651 000	64.549	15
» 2.	125.250	2.784.775.050.000	14 298 700	194.756	5
» 3.	104	5 229 126.108.000	11 774.025	444 123	2
» 4.	151.700	669 801 285 000	5 736.732	116.755	8
» 5.	142 500	7.743 706 142 000	8 100 375	955.967	1
» 6.	133	6 527.707 536 000	1.388 124	4.702.538	0 2
» 7	201	1.104.286.623.000	2.199 748	502.004	2
» 8	128.750	6.186 609.671 000	723.714	8 548.417	0 1

Finally we come to the last question, of great signification for the physiological bacteriology of the intestinal canal: Have the micro-organisms of the intestinal canal of the rabbit to play a part in the digestion?

If attention is paid to the following facts:

1°. The very small number of living bacteria with respect to the number of grams of intestinal contents;

2°. The very small number of living bacteria with regard to the number of dead ones, in particular perceptible from the high sterility-indices of the whole intestinal canal, and from the slight number of living bacteria found on 1 million of dead organisms, and

3°. That at no single place there is a multiplication, on the contrary, that nearly in the whole intestinal canal there is a mortality on large scale of living bacteria, we are obliged to deny the bacteria playing any part in the digestion in the intestinal canal of the rabbit.

Mathematics. — „*On the motion of variable systems*" by Prof. CARDINAAL.

1. With considerations relative to the theory of motion, we generally start from the principle that two phases of the system are congruent. If the two systems considered in this way are situated in a plane the pole of the motion is the only real point of coincidence of the two systems; if they are situated in space the principal axis of the motion is their line of coincidence. If we suppose the second system to have approached the first at infinitesimal distance, the rays connecting the homologous points are directions of velocities and one of the principal problems of motion consists of the construction of the directions of these velocities. Special constructions exist for this, the second system not being suitable for use.

2. In the plane the construction of the direction of velocities is a simple matter, the polar rays being normals to the orbits and the velocities touching them. In space the construction becomes already more elaborate; however, we can notice that the directions of velocities are the rays of a tetraedral complex; to this complex belongs moreover a focal system, the properties of which enable us to find the points belonging to these rays. This paper now purposes to investigate this same subject for systems of points in space changing projectively during their motion. The investigation is independent