of Caspar Bauhin, the Spiceligium of Theodore Kerkringh, the Encheiridium Anatomicum of Riolan, and the Alkmaar Pharmacopœia.

The price is $1.50. We are informed that it is possible to obtain the pictures of the preceding calendars, together with albums for holding them, at a cost of eight shillings and sixpence each, or the twelve albums for five pounds, on application to the publisher. The calendar is beyond question an unusual and artistic production. A. G. N.

ROYAL COLLEGE OF PHYSICIANS AND SURGEONS OF CANADA

The Royal College of Physicians and Surgeons of Canada desires to draw to the attention of the physicians and surgeons of Canada, Section 5 of the Act of Incorporation of the College, which Section reads as follows:—

> "The Council may, at any time within two years after the coming into force of this Act and without examination, select and admit as Fellows any duly qualified persons domiciled in Canada who have in their opinion given evidence of high ability in one or more departments of medicine." (Act assented to and in force June 14, 1929.)

Members of the profession who consider themselves as qualified for admission to Fellowship under this Section of the Act, are invited to apply for qualification forms to Dr. T. C. Routley, Registrar-Secretary of the College, 184 College Street, Toronto.

Special Articles

THE ELECTROPHORESIS OF NEISSERIA INTRACELLULARIS (MENINGOCOCCUS)

A PRELIMINARY REPORT*

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This paper is more of the nature of a preliminary report, presented at this time with the hope that it may suggest a new method of attack on the problems of determining in the laboratory the relative virulence of strains of meningococci and of the potency of antimeningococcus sera. Since the whole field of meningitis has been so completely reviewed from all angles many times, only those references which are pertinent to the contents of this paper will be quoted.

Meningococcic meningitis is to be considered an extremely important public health problem at the present time. Dr. McCoy, of the Hygienic Laboratories, Washington[1] states that "More serious outbreaks have occurred in the past year than since the world war." Dr. M. J. Rosenau[2], in his book states, "It is not clear that any of the measures so far taken have either materially influenced the course of epidemics or prevented the spread of the disease." These statements made by outstanding authorities serve to emphasize the importance of the problem.

The recognized method of treatment of meningitis is by the use of anti-meningococcus sera. This is a specific treatment and if there were a laboratory method of measuring potency in terms of therapeutic efficacy the treatment of cases of meningitis would be relatively simple. However, as Topley and Wilson[3] state, "As yet there is no laboratory method by which the therapeutic power may be correctly gauged." Further evidence indicating how serious this phase of the problem is becoming has been presented in American literature on numerous occasions within recent years. Norton and Gordon[4] report an epidemic in Detroit involving 867 cases. Of these 430 died, giving a case fatality rate of 53 per cent. They concluded their report by stating "It must, therefore, be concluded that the strain of meningococcus encountered was of unusual virulence, or that the therapeutic serums available were not specific against this strain."

All of the immunological tests have been applied in an attempt to measure potency. At the present time the agglutination test is the most widely used, but it is recognized that this test serves only to indicate that the horses have been injected with certain standard strains of meningococci. In fact, it has been reported that antisera of the lowest agglutinin content have been more efficacious than others of high agglutinin titre.

Before attempting to measure the potency of anti-meningococcus sera there should be a laboratory method of determining the relative virulence of strains of meningococci. However, such a method does not exist, largely because there is not a common laboratory animal susceptible to meningitis. At the present time cultures are carried on media varying widely in composition in different laboratories and these cultures, ten to fifteen years old, are used not at all infrequently for injecting horses for the production of antisera. It is a well known fact that cultures of other organisms lose their virulence, and in some cases pathogenicity after prolonged culture in the laboratory. It would, therefore, be logical to assume that meningococci may behave in a similar manner.

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* A paper read on August 28, 1930, at Winnipeg, in the Section of Public Health, British Medical Association. From the Laboratory of Hygiene, Department of Pensions and National Health, Ottawa, Canada.

The determination of virulence of strains of meningococci is extremely difficult. The situation is adequately summarized by Murray[15] who writes as follows:—

"The determination of the virulence of cultures has been admitted by most authors to be extremely difficult, and the results have been so inconstant from one experiment to another, although the same strain has been used, that it has been held to be impracticable (Dopter 1921). One of the most important factors upon which success depends, both for the titration of virulence and for its maintenance in cultures, is the constitution of the medium (Murray and Ayrton, 1924). In experiments in which all other known factors have been kept as nearly as possible comparable, controlled variation of the concentration of tryptic digest in the medium has resulted in the cultures being highly virulent on one and harmless on another medium."

Gordon[5] believes that "too much weight must not be laid on the results obtained with laboratory specimens, as experience has shown that meningococci under prolonged culture on egg and trypagar media may tend to become more highly specialized than when recently isolated."

Gordon[6] attempted to use endotoxin as a measure of virulence, but this method has not been generally accepted. Murray[15] states "The exact relation of virulence to endotoxin is still uncertain, but the available evidence tends to show them to be unrelated (Gordon, 1920, p. 45; Murray, 1929). Strains of low virulence with a high yield of endotoxin are found, but more commonly a low yield of endotoxin is associated with a high or medium virulence. Exalting or lowering of virulence does not appear to alter the quantity of toxin yielded by a strain but only affects the power of infecting animals."

In the light of the work by Gordon and Murray on the effect of culture media on virulence, it is possible that by careful selection media have been developed in certain laboratories which will maintain virulence and that this fact may also account to some extent for the variation in clinical results with different antisera.

Another difference frequently noted is that some freshly isolated strains are inagglutinable, but after cultivation for a time in the laboratory they agglutinate perfectly. The author has knowledge of one very striking case of this kind. A patient died within twenty-four hours from fulminating meningococcic meningitis. The organism isolated from the spinal fluid would not agglutinate with any sera, but after cultivation for approximately three months, it agglutinated perfectly. It must be assumed that this strain changed its characteristics during these three months' cultivation on artificial media.

The one point upon which all workers seem to agree is that meningococci are very unstable in their characteristics, and that it is very difficult to produce consistent results. Such a simple procedure as transferring the cultures on stock media frequently results in poor or negligible growth, even though the same batch of media is used under identical conditions. Cultures will suddenly die out for no accountable reason. Taken by and large, the group is very poorly understood, especially in relation to its immunological phases.

Since the ordinary immunological tests have failed to give any valuable information as to the virulence of the meningococcus, or the potency of antisera, it was thought that a study of the behaviour of the meningococcus in an electrical field (electrophoresis) may yield some further data. It at least offers a new method of attack.

Jensen and Falk[7], have shown a distinct correlation between the virulence of the diphtheria bacillus and the rate of migration in an electric field (electrophoresis). Similar correlation has been obtained by Falk, Jacobson and Gussin[8], with a pneumococcus. Kahn and Schwarzkopf[9] have determined the rate of migration in an electric field of the rough and smooth strains of tubercle bacilli, but do not yet wish to convey the impression that there is also correlation with virulence.

Mudd, Lucke *et alii*[10] have shown that phagocytosis of bacteria is correlated with the potential charge,* *i.e.*, particles of a high charge are not phagocytized, but when the charge is lowered, *i.e.*, by the addition of serum, they are readily engulfed.

Murray[11], states, "As the virulence of a strain (meningococci) is increased, it appears to become more resistant to phagocytosis although this resistance is lost when the cocci are killed."

Hiss and Zinsser[12], state in reference to meningitis, "A certain amount of prognostic information can be obtained from spinal fluid in that in severe cases that are not doing well there will be a considerable number of organisms, extracellular."

Kolmer[13], says, "As the case improved, whether under serum treatment or spontaneously, the micro-organisms diminish in number and become intracellular."

When these various statements are analyzed it would appear that if, as Mudd, Lucke and others hold, phagocytosis is a function of the potential charge, then the strains of meningococci found extracellularly may have a higher potential charge than those found intracellularly, and, conversely, those found intracellularly may have a lower potential charge. A quotation from Falk[14], may be pertinent at this point: "I am persuaded that the older notions on virulence and its mechanism have not been fruitful; a new one, which for the moment places the dynamics of electrical potential differences as a focal point between 'virulence' and 'resistance', may not be more sound, but it cannot be more sterile." The

*Potential charge is the electric charge calculated from the formula of Helmholtz and Lamb from the observed velocity of migration of a particle in a measured field and under a known difference of potential. It is considered advisable to express the velocity of migration in terms of μ per second, per volt, per centimetre, since the accuracy of the equation is in doubt.

following is an account of my own researches into this problem.

THE MEASUREMENT OF POTENTIAL

The potential was determined from the velocity of migration with a Northrup-Kunitz micro-cataphoresis cell.

The fall in potential in the cell was determined at the beginning and end of each experiment by the "null" method described by the author[16]. The rate of migration was determined by observing the movement of the bacteria at the "stationary layers", as determined from the formula $\frac{1}{\sqrt{3}}\frac{D}{}$ above and below the middle of the cell. Ten readings were made at each level, five with reversed polarity. The impressed voltage was 97+ 1. Since there is some doubt as to the correctness of the Helmholtz-Lamb equation, the results are expressed in μ per second per volt,, per cm., as well as in terms of millivolts.

BUFFERS

Walpole's acetic acid-sodium acetate mixture at pH 5.4 $\frac{M}{1000}$ concentration was used throughout the work. The pH was determined colorimetrically.

AGGLUTINATION METHOD

The macroscopic method as outlined by the Hygienic Laboratories, Washington, was used.

CULTURES

Unless otherwise stated, all cultures were used when 24 hours old. For the determination of rate of migration all cultures were heated at 65°C for 1 hour and washed three times in $\frac{M}{1000}$ acetic acid-acetate buffer without any other salts.

MEDIA

Glucose beef infusion agar at pH 7.6 was used unless otherwise stated.

EXPERIMENTAL

In the early part of the work reported herein it was found that washed suspensions of meningococci in the presence of sodium chloride were very unstable. Various concentrations of salt were used in an attempt to find one which would not cause spontaneous agglutination and yet be sufficient for the ordinary agglutination test, but without success. It was therefore decided that for the first part of this study it would be advisable to suspend all organisms in $\frac{M}{1000}$ acetate acetic acid mixture at pH 5.4 for measurements of velocity of migration.

The points to be particularly noted in Table I are as follows:—

1. Determinations listed under column A were made on cultures which had been carried for several weeks on media with a reaction of pH 6.8. This acid reaction was found to be due to hydrolysis of dextrose by prolonged sterilization. It was therefore decided to add sterile dextrose under aseptic conditions in order to maintain a reaction of pH 7.6. Consequently the cultures used for the determinations in column A had only been transferred once or twice on the new

TABLE I

Potential Difference of Strains of Meningococci

Culture N°	Culture Type	Source	Probable Date Isolated	A P.D.	A μ Sec V Cm	B P.D.	B μ Sec V Cm	B' P.D.	B' μ Sec V Cm	C P.D.	C μ Sec V Cm
1	I	H.L.	1917	27.4	1.99	31.7	2.27			32.3	2.32
3	I	Conn.(H.L.)	1917	30.8	2.24	30.6	2.19			28.7	2.06
7	I	N.Y.S.	1916	31.9	2.32	39.2	2.81			38.6	2.77
8	I	N.Y.S.	1918	37.1	2.70	39.2	2.81			38.5	2.76
12	I	H.L.	old	31.5	2.29	39.2	2.81			37.3	2.68
32	I	Chicago	Apl 4/30			40.1	2.87			36.1	2.57
2	II	Conn.(H.L.)	1917			32.2	2.34			30.6	2.19
5	II	N.Y.S.	1916			28.6	2.08			31.6	2.26
6	II	N.Y.S.	1916			33.5	2.44			30.9	2.22
15	II	H.L.	old			23.2	1.69			24.2	1.77
16	II	H.L.	1917			34.9	2.54			35.1	2.52
9	III	N.Y.S.	1916			38.4	2.87			38.5	2.74
10	III	N.Y.S.	1918							37.4	2.67
19	III	H.L.	Dec 16/28			37.1	2.70			38.0	2.71
28	III	Chicago	July 2/30			36.6	2.66	33.8	2.42	32.0	2.28
33	III	Chicago	Apl 1/30					33.6	2.41	31.7	2.26
4	IV	Conn.(H.L.)	1917	30.8	2.24					26.8	1.92
20	IV	H.L.	May 14/28	29.0	2.11					25.6	1.85
21	IV	H.L.	March 28/29	29.3	2.14					28.5	2.04
22	IV	H.L.	May 14/28			30.2	2.20			30.5	2.18
27	IV	N.Y.C. H.D.	June 28/30			36.0	2.62			41.5	2.96
31	IV	Chicago	Apl 29/30			38.2	2.78			36.4	2.60
34	IV	Detroit	July 4/30					41.9	2.99	38.3	2.73
38	?	Kingston	July 21/30 Case					38.9	2.77	34.8	2.48

media. Those determinations listed under columns B, and C, were made ten days to a month later and show close agreement with each other but considerable variation with column A. This point is significant in that it indicates that changes in the media are accompanied by changes in potentials and is in line with Gordon and Murray's contention that the constitution of the media is extremely important.

2. It will be noted, especially in Type IV strains, that those cultures isolated in 1930 have a potential difference (P. D.) of from 36.0 to 41.9, while the old strains vary from 25.6 to 30.5. This fact may indicate measurable difference between freshly isolated and old stock strains of meningococci. This difference noted in Type IV is not found in the other groups, probably because sufficient new strains were not obtainable for comparison.

3. Another fact which is brought out rather strikingly is the great variation in P. D. between strains of one group. This variation is entirely in line with other characteristics of meningococci. It is rather interesting to note that cultures No. 2 and 16, supposed to be the same strain, were obtained from different laboratories and were undoubtedly carried on different media.

Table II presents an analysis of the data in a different manner. It shows that the range in P. D. covered by the old strains of each type show considerable overlapping, but with Types III and I higher than II and IV. Chart 1 further emphasizes this point.

TABLE II.

COMPARISON OF POTENTIAL DIFFERENCE OF OLD AND NEW STRAINS OF MENINGOCOCCI

Old Strains					*New Strains (First Test)*				
Type	*No. of Strains*	*P. D.*			*Type*	*No. of Strains*	*P. D.*		
		Average	*Low*	*High*			*Average*	*Low*	*High*
I.	5	35.08	28.7	38.6	I.	1	....	....	40.1
II.	5	30.5	24.2	35.1	II.	2	35.1	33.6	36.6
III.	3	38.0	37.4	38.5	III.		....	....	....
IV.	4	27.8	25.6	30.5	IV.	3	38.7	36.0	41.9
					?	1	....	....	38.9

Charts 2 to 8, inclusive, present the data in different form and serve to emphasize certain variations. Chart 2 shows the extreme variation between strains of one type and at the same time indicates the tendency to type variation. Charts 3, 4, 5 and 6 are self-explanatory.

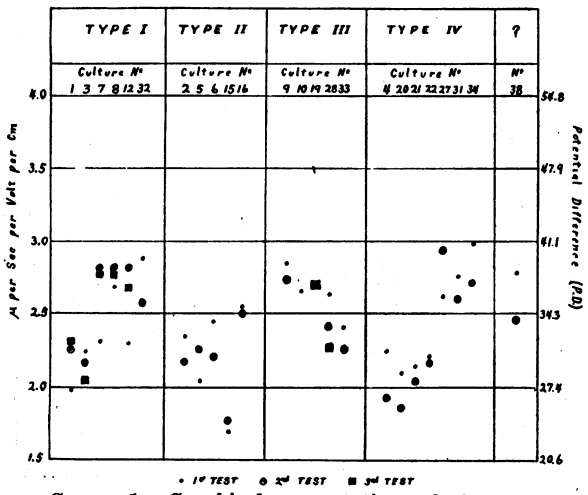

CHART 1.—Graphical presentation of the data in Table I.

Charts 7 and 8 compare the P. D. of strains isolated in July and April, 1930. It will be noted that the P. D. of July strains show a very sharp initial drop after a few subcultures, while April strains are much more stable. Just how significant these data are, is difficult to say, but it is very suggestive that according to determinations of P. D., recently isolated strains have a higher charge and tend to lose it rapidly.

Preliminary investigation has shown that the charge reducing properties of specific antisera are measurable by determining the P. D. of treated and untreated cocci.

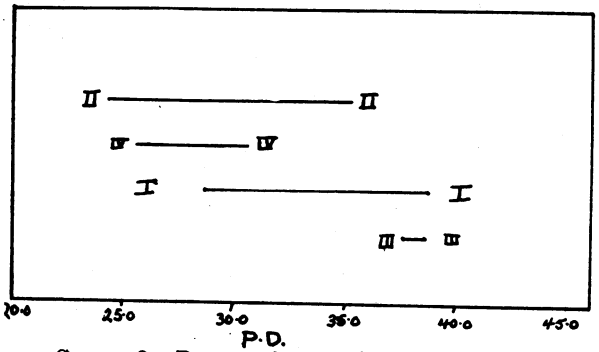

CHART 2.—Range of potential difference of old strains of meningococci (see Table II).

CONCLUSIONS

In conclusion it may be stated that it appears from the work thus far, that the determination of the potential difference (P. D.) of strains of meningococci may open up a new method of attack on the problem of determining their relative

virulence. Evidence has been obtained that at least for Type IV strains, those recently isolated (July, 1930) have a distinctly higher potential than the old stock strains. It is further indicated that the variation within a group of meningococci of one type may be greater than between different types.

The significance of these variations in P. D. and their bearing upon virulence of strains of meningococci and the production of potent antisera is problematic. The fact that there are definitely measurable differences in P. D. is encouraging, especially since it is known that other organisms vary in virulence from one extreme to another.

Certain definite lines of research are indicated:—

1. Extension of the results reported herein.
2. The development of media which will maintain the high charge on freshly isolated cultures.

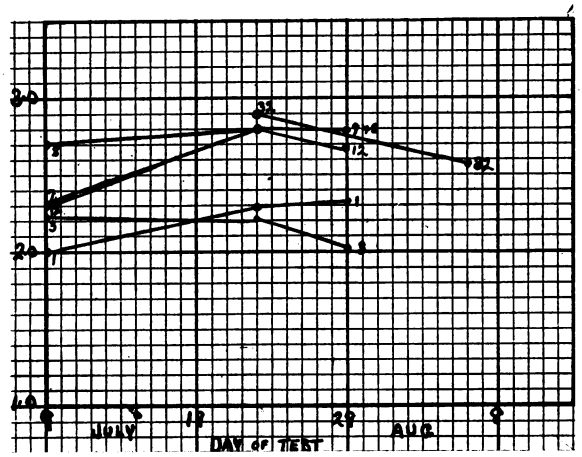

CHART 3.—Type I Meningococci.

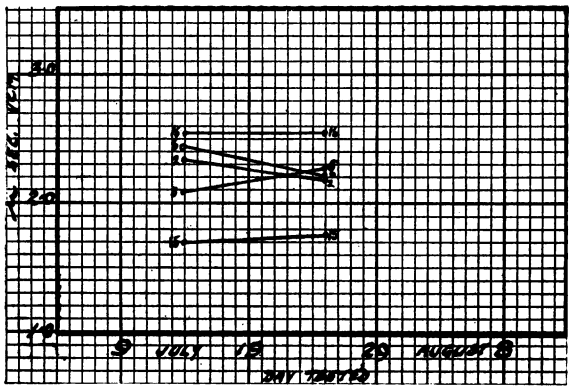

CHART 4.—Type II Menginococci.

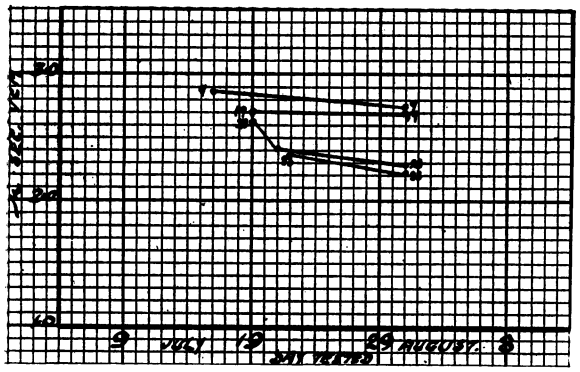

CHART 5.—Type III Meningococci.

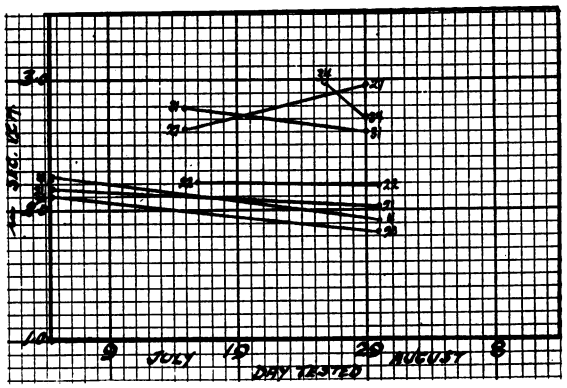

CHART 6.—Type IV Meningococci.

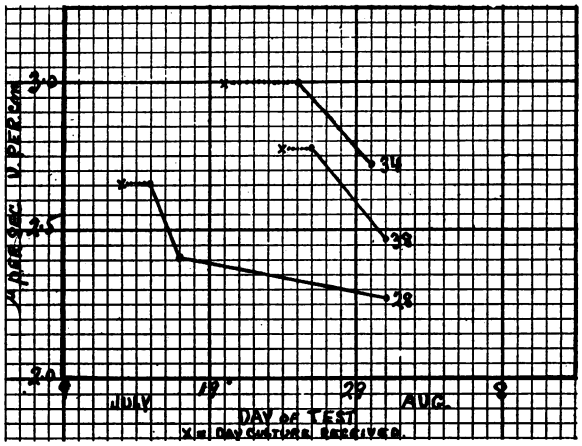

CHART 7.—Meningococci isolated in July.

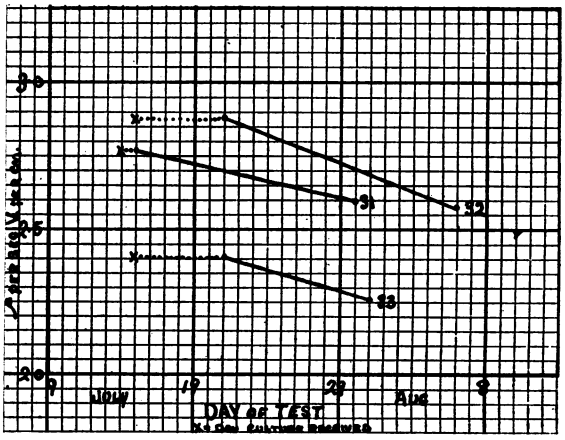

CHART 8.—Meningococci isolated in April.

3. The determination of the P. D. of strains of meningococci isolated from spinal fluid and from carriers. Notation as to whether or not spinal fluid strains were found extracellular or intracellular should be made.

4. The determination of the phagocytic index of strains with a high charge as compared with those of low charge.

5. The charge reducing effect of various antisera.

6. The production of antisera by the use of strains of meningococci with a high charge and the careful clinical observation of results.

Many other suggestions could be offered but these few will serve to indicate the possibilities in this field of research.

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Men and Books

MEDICAL MATTERS IN THE APOCRYPHA*

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Much has been written on matters medical and hygienic which are contained in the canonical scriptures, especially on the hygiene of the Pentateuch, but a similarly careful study has not been made of these subjects in the Apocrypha. Yet some of the directions and declarations in "Ecclesiasticus" and in "Wisdom" are more specifically "medical" than even the famous sanitary laws of Moses.

To begin with, the author of "Ecclesiasticus", Jesus, the Son of Sirach, of Jerusalem, speaks with no uncertain voice in praise of the physician. —"Honour a physician with the honour due unto him for the uses which ye may have of him, for the Lord hath created him," (xxxvii., v. 1) so that on a literal acceptation we might claim "the divine right of physicians" on better grounds than crowned heads have claimed the "divine right of kings."

"The physician cutteth off a long disease," we are told in chapter x., v. 10. But these are only the introductions to his high opinions of our profession, for in chapter xxxviii., v. 4, we have—"The Lord hath created medicines out of the earth, and he that is wise will not abhor them." Unquestionably the "medicines" referred to here are herbs and not what we should now call chemical or mineral substances. Therapeutic materials of non-vegetable origin were almost never prescribed before the time of Paracelsus (d. 1541). It is, however, true that in ancient Egypt some mineral substances were used, but mostly for cosmetic purposes.

The salutary effect of a medicine is used as a simile for a faithful friend—"a faithful friend is the medicine of life" (vi., v. 16). The prophylactic aspect of therapeutics is given in a picturesque parallel—"Learn before thou speak, and use physic or ever thou be sick" (xviii., v. 19). We venture to think that this latter aspect of medicines as preventive was not one prominently before even thoughtful people at the date of the "Ecclesiasticus."

Jesus, the son of Sirach, had also respect for the apothecary. He speaks of the art of the apothecary in almost extravagant terms of praise ". . . . . the composition of the perfume that is made by the art of the apothecary; it is sweet as honey in all mouths, and as musick at a banquet of wine" (xlix., v. 1). This simile of music at a banquet is a favourite with the author of "Ecclesiasticus"; he uses it more than once, as we shall see. The value of sound general health is emphasized in several passages. Thus, "Health and good state of body are above all gold, and a strong body above infinite wealth" (xxx., v. 15); and again—"Better is the poor being sound and strong of constitution than a rich man that is afflicted in his body" (xxx., v. 4). Many a rich invalid has said "Amen" to this. Once again—"There is no riches above a sound body" (xxx., v. 16).

*Scholars are not unanimous in regard to the date at which "Ecclesiasticus" in its original Hebrew was written. Some assign it to the third century B.C., others to a century later. The most generally accepted opinion is that it was written by a Jew, Jesus of Jerusalem, whose grandson, Jesus, son of Sirach, of Jerusalem, translated his grandfather's treatise into Greek and added a prologue of his own in that language.

(Cf. "The Books of the Apocrypha," by the Rev. Prof. Ryle, B.D., in "The Cambridge Companion to the Bible," Cambridge, 1894.)