A CLINICAL METHOD OF MEASURING THE ANTITRYPTIC INDEX,

ILLUSTRATED BY ITS RESPONSE TO VACCINES.

BY

FRANK C. EVE, M.D.CANTAB, M.R.C.P.LOND.,

PHYSICIAN, ROYAL INFIRMARY, HULL.

THE object of this investigation has been to devise a method of measuring the antitryptic index suitable to ordinary clinical work, and applicable to the regulation of the size and frequency of the doses of vaccines. The method will be illustrated by a chart showing the daily variations in the index during a period of three months in a case of pyelonephritis (with relapse) treated by tuberculin and *coli* vaccine.

A few introductory remarks may be helpful. If a viscous solution of casein is incubated with trypsin, digestion of course rapidly occurs, and is manifested by the loss of viscosity of the mixture. This may be measured by the viscosimeter—that is, a pipette with a fine capillary stem of thermometer tubing. If now a drop of ordinary blood serum is added the rate of digestion is enormously retarded, and if the serum has been taken from a patient suffering from cancer, tuberculosis, or any inflammatory or cytolytic disease, the rate of digestion will be still more powerfully inhibited. Hence if two mixtures of similar quantities of casein, trypsin, and serum (one normal, the other from the patient) are digested for 4½ hours, and the number of seconds that each takes to drip through a viscosity pipette is compared, the two numbers will give a measure of the antitryptic power of the patient's blood. Supposing the normal mixture drips through in 100 seconds and the pathological mixture in 103½ seconds, then the antitryptic index may be charted as 3½.

This method of estimating the relative antitryptic values of different serums, based on Bayliss's well-known method of estimating the velocity of tryptic digestion by the viscosimeter, was devised by Dr. Golla and demonstrated by him at Belfast[1] in July, 1909. This observer had also successfully measured the relative powers of normal and pathological serums by an adaptation of Victor Henri's method of electro-conductivity, as also had Dr. E. C. Hort[2] by adapting Sörensen's method of measuring the degree of amino-acid liberation during a tryptic digestion.

Both of these methods have been shown by these observers to give excellent results, but they are both of them complicated in apparatus and procedure, and the chemical method has the additional disadvantage that it demands more blood than can readily be obtained from a finger-prick. Now, to obtain several cubic centimetres of blood from a vein is a very neat and painless process when properly done, but it cannot be repeated often, and sometimes makes the patient feel faint and queer, as I can testify from personal experience. To the patient, the value of the information gained may make this worth his while, but to get an equivalent supply of normal blood is often a matter of serious practical difficulty. I have selected, therefore, the viscosity method, though I found that the technique required considerable modification in order to adapt it to clinical purposes.

After experimenting for four months, I found that by digesting for four and a half hours in the incubator instead of at room temperature, and by adding another 3 c.cm. of fresh casein just before measuring the viscosity, the antitryptic power of a serum could be reliably measured with only 0.08 c.cm. of serum and with quite homely apparatus.

Method.

Ordinary glass tubing is washed with acid and then with water and dried. It is then drawn out at intervals of 3 in., whereby a number of cylinders with tapered ends are made. If held horizontally in the drop of blood on the patient's finger, these fill themselves without forming bubbles. The finger must be warm and preferably should be swung round the head before applying the tourniquet of rubber tubing.*

*I find the best pricker is made by pounding some sheet glass, picking out the sharpest fragments, mounting each with sealing-wax on to a glass rod, which is fixed into the cork of a small phial containing a little wool moistened with formalin. These are clean and painless, and last for weeks.

The tubes, when sealed, are put for an hour into the incubator to contract the clot, after which they may be kept for several days at the temperature of tap water without alteration of the index.

Casein Solution.—This is made by weighing 6 grams of casein (prepared according to Hammarsten) and stirring it into a paste with 30 c.cm. of distilled water warmed to about 40° C. After adding 5 c.cm. of decinormal NaOH, the mixture is stirred till the particles are all dissolved with the help of five drops of liq. amm. fort. Mix this solution with 95 c.cm. of water, to which five drops of formalin have been added, and filter through paper (or wool) carefully. Repeat the filtration every day the solution is used, as a glutinous precipitate soon forms.

The Piston Pipette (Fig. 1).—I should be very sorry to dispense with the help of this device, adapted from the design of my friend, Mr. Upcott. It consists of a home-made capillary pipette with a little bulb, B, holding about 0.1 c.cm. up to the mark, ↑, which may conveniently be made of a filament of cold sealing wax laid across the hot glass. The butt end of the pipette slides freely inside a larger glass tube, and the piston-joint is

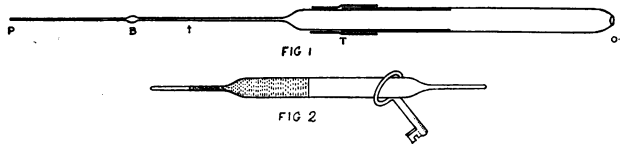

Fig. 1.—Piston pipette for measuring small quantities of serum. It consists of two tubes sliding one within the other, with a connecting collar of rubber tubing, T. One tube terminates in a capillary and a small bulb, B; the other in an orifice, O. Drawn one-third natural size.

Fig. 2 illustrates the use of a small key as a lever in breaking a blood capsule at the file mark without the risk of breaking the capillary ends. One-third natural size.

made tight by a collar of rubber tubing, T, fitting snugly, and lubricated by a drop of water. A finger over the hole, O, converts it into a syringe, but the moment the finger is removed the internal air pressure is equalized, and the contained fluid (if held horizontally) remains stationary. Final accuracy is obtained by removing any slight excess of fluid, as a succession of dots on the finger which is repeatedly tapped against the point, P. Alternatively the hole, O, can be applied to the mouth, and fluids can be aspirated or expelled by suction, as when washing it out.

Trypsin Solution.—Four grains (0.26 gram) of Armour's trypsin are stirred into a paste and mixed with 100 c.cm. of water which has been freshly drawn from the tap to secure uniformity of temperature. The solution is filtered twice, and must not be prepared till just before use, as it rapidly deteriorates in the absence of substrate.

The chart was made by collecting the blood daily and keeping it in the cool till the fourth day, when the antitryptic power was measured against one normal serum (my own). Thus the estimations were done in batches of five, and hence five similar short test tubes were required.

Into each 5 c.cm. of casein solution are measured; 0.08 c.cm. of serum (approximately) is then very exactly measured into a capillary pipette (Fig. 1), which is rinsed out thrice into the test tube of casein. The pipette is then rinsed out four times in water and dried ready for the next serum by toasting it in front of a gas fire, expelling the steam by blowing, and cooled by sucking in cold air. The point may either be plunged into the serum, or if scanty it is best to open both ends of the capsule and pour the serum from capsule to pipette held mouth to mouth. It is often difficult to break the blood capsules at the thick part without breaking the capillary end. If the handle of a little key is slipped over and used as a lever the tube will always break at the file mark (Fig. 2).

Each casein-serum mixture is shaken up as soon as it is made, and every test tube is conspicuously marked—for example, N, P_1, P_2, P_3, P_4—with a grease pencil; 1 c.cm. of trypsin is now added by pipette to each test tube. All five test tubes are corked, shaken, and incubated for four and a half hours at about 40° C. They are then cooled under the tap and allowed to stand until next morning, if, as usually happens, this is most convenient.

Measurement of Viscosity.

Five little flat-bottomed, steep-sided glass cells are required, about 2 in. in diameter and 1 in. high, which can be covered with a watch-glass and immersed in a photographer's developing dish (to act as a water bath). They are numbered in correspondence with the five test tubes; 3 c.cm. of fresh casein solution are now added to each test tube, and the mixture is shaken and poured into each dish. The fresh casein is added because I

found that acid decreases the viscosity of casein, and hence the amino-acids accumulated during the four and a half hours' digestion will accentuate the difference in the readings of the viscosimeter, and thus render small differences in the antitryptic activity of the serums more measureable. Also the serum will be acting in a state of greater concentration.

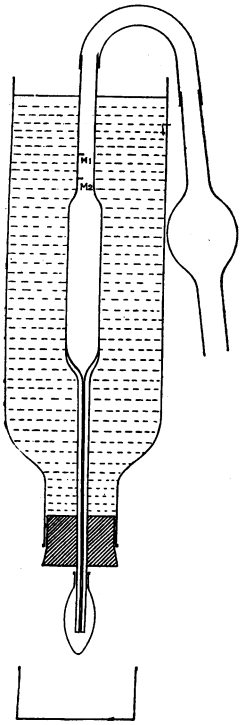

Fig. 3.—Viscosimeter, consisting of a pipette surrounded by a water bath, for measuring viscosity. It consists of a capillary tube surmounted by a bulb holding about 7 c.cm. This is connected by rubber tubing with a glass bulb which conveniently converts intermittent efforts of suction into continuous suction. The water bath is simply an inverted pint bottle with the bottom cut off. Below a glass capsule is shown in section. A rubber teat protects the capillary orifice from drying when not in use. The drawing is one-third natural size.

The five dishes now contain 6 c.cm. of casein-trypsin-serum digest *plus* 3 c.cm. of fresh casein. When they have stood for an hour or two to attain a condition of temperature equilibrium with their surroundings—very important—each solution is aspirated in turn into the viscosimeter and the number of seconds which each fluid takes to run through from the mark M_2 to the top of the capillary portion (see Fig. 3) is measured with a stop-watch. Each observation is repeated twice, or more if not concordant. The start should be a flying one from the mark M_2, starting from rest at the mark M_1.

Owing to the marked effect which temperature has on viscosity, the laboratory must be kept at a constant temperature during and before the observations. The whole apparatus should be considered as immersed in the air of the laboratory as in an air bath, so that a separate room is desirable. The observer must not touch the glass dishes with his warm hands, and the heat of his body must not be allowed to raise the temperature of the bath surrounding the pipette by more than 0.5° F. during the five observations.

If the temperature of the air is inconstant, a lower water bath may be necessary; the developing dish (or circular tray of perforated zinc) stands supported on a block inside it. Bits of fluff must on no account be allowed to get into the digest, and bubbles of air which have a way of clinging to the mouth of the pipette will surely vitiate a reading. But if these troubles be avoided the readings will with practice become very concordant, and should not differ by $\frac{1}{2}''$.

The protocols are tabulated thus:

G.H.—Serums of April 18th, 19th, 20th, 21st.

Time.	Normal.	Patients.				Temperature of water bath.
		No. 1.	No. 2.	No. 3.	No. 4.	
1.30 a.m. ...	73.2″	74.0″	74.7	75.2	72	Before, 53° F.
	73.2″	74.1″	75.2	75.4	72	After, 53° F.
	73.0″	—	74.7	75.3	—	
Difference	—	+0.9″	+1.5	+2.1	−1.2	
Add 27% ...	—	+1.1″	+1.9	+2.6	−1.5	

I add 27 per cent. here to bring results to a constant normal of 100″, so as to make the different parts of the curve (which has a fresh normal every four days) comparable. If the normal had come out at 70″, for instance, I should have added 30 per cent.

The results are plotted on the curve shown (Fig. 4), which is probably unique in being a daily record of the antitryptic index in an acute relapsing illness treated with vaccines during a long period.

In sucking up the serum into the measuring pipette a proportion of red cells will often be included. The mixture

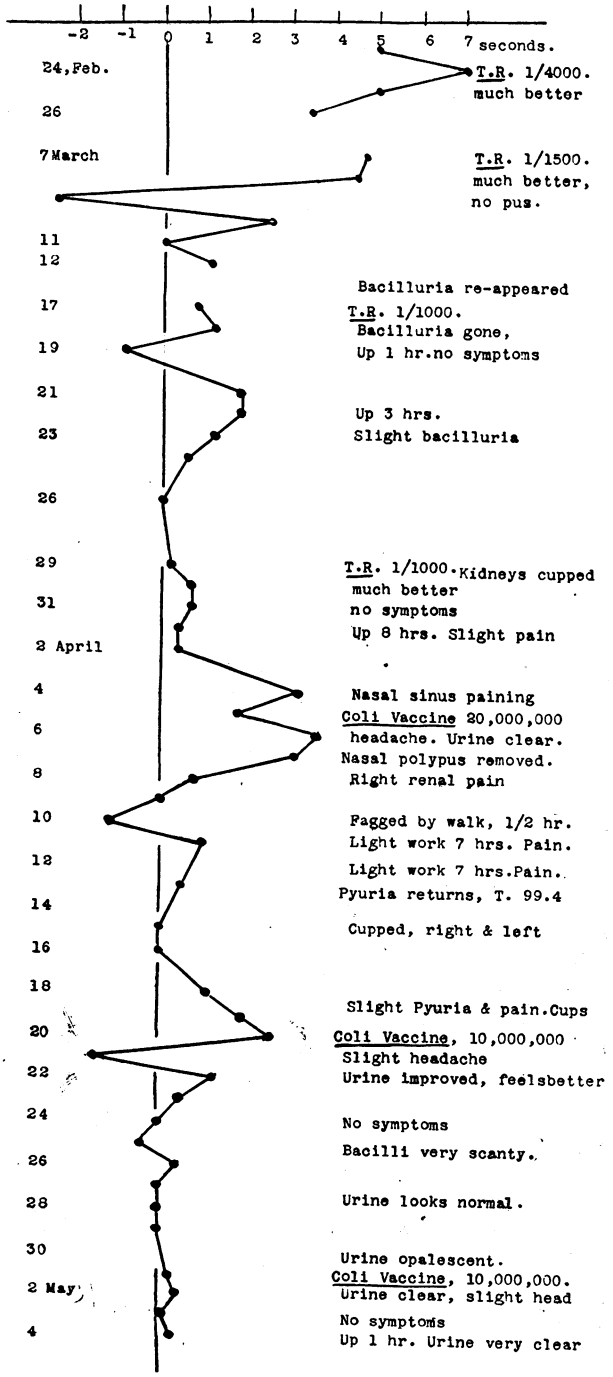

Fig. 4.—Chart showing the daily variations of the antitryptic index during two and a half months in a case of pyelonephritis treated by tuberculin (four injections) and *coli* vaccine (three injections).

has, I think, a slightly less antitryptic power than pure serum, but side experiments indicate that the effect is negligible in slight degrees of red cell contamination.

Illustrative Case of Pyelonephritis Treated with Vaccines, Showing Their Effect on the Antitryptic Index from Day to Day.

The patient (female, aged 27) showedi n her history a remarkable susceptibility to bacterial invasion. At the age of 12 she very nearly died of tuberculous peritonitis, but her natural desire to disappoint her schoolfellows in the band (whom she heard practising the Dead March for her benefit), doubtless contributed to her recovery.

Next came slight tuberculous glands, continuous amenorrhoea, and tuberculous disease of the bone and skin of the great toe, which, after thirteen operations, yielded to amputation.

Then she developed a persistent nasal catarrh, from which I isolated the bacillus of Friedländer, and prepared a vaccine which did temporarily stop the rhinorrhoea, but did more harm than good. A nasal polypus was then removed, and was followed by infection of the maxillary antrum. This was drained and washed, but the other sinuses became infected one after the other. All the sinuses have now been operated upon except the sphenoidals, and now, by wearing a tiny style to drain the frontal sinus, the poor girl seems to have been given relief from this trouble. However, after each operation urethral catheterization became necessary, and at the last one a cystitis due to *B. coli* infection was acquired in spite of every precaution. It spread up to the kidneys, causing a critical illness (February 6th; 1910).

The chart commences two weeks after this had passed its acute stage, while there was still pain and tenderness in the kidneys, and much pus in the urine. Though the temperature was normal, the antitryptic index was high (7″ delay). I did not dare risk the possibly injurious effect of a *coli* vaccine at this stage, though if one could have foreseen how obstinate the case would prove, a vaccine would have been worth the risk. As she had previously reacted very well to tuberculin (it always acted like a strong tonic) I gave her a small dose of T.R. ($\frac{1}{4000}$ mg.).

This and three other doses had an unmistakably good effect on all her symptoms, and also on the antitryptic index. A relapse, however, occurred, due partly to the necessity of her having another nasal polypus removed. This probably caused the big rise in the index about April 4th. The dose of *coli* vaccine she then had was probably slightly too large (20 million), as it produced a preliminary rise in the index and a fall delayed too long. The dose on April 20th was probably correct, as it caused a prompt and decisive fall in the index (4″), but of course one cannot generalize on such slender evidence. A vaccine appears to cause no fall of a normal index, but too large a dose would no doubt produce a rise, so that in either case the index may afford valuable guidance.

Those who are accustomed to opsonic curves must bear in mind that our object is to coax the curve *down* to the normal instead of trying to force the curve *up*, as in opsonic work.

Dry cupping I found of great benefit in this and similar cases, but I could not disentangle any effect on the antitryptic index attributable to it.

Urotropin, as usual in my experience of a *coli* urinary infection, did no good, but helmitol, as usual, proved distinctly helpful.

Methylene blue did no good in this case, and possibly harm.

The index varied independently of the temperature, proving much more sensitive to the vagaries of the kidneys and to the influence of vaccines.

CONCLUSIONS.

1. It is possible by this method to measure the antitryptic index with only $\frac{1}{12}$ c.cm. of serum, which is easily obtainable from a finger-prick.[1]

2. It appears probable that repeated observations of this index may give valuable guidance in the amount and frequency of doses of vaccines.

3. A suitable dose apparently lowers a raised index sharply without a preliminary rise (vide chart, March 8th and April 21st).

4. A dose which is unduly large seems to cause a preliminary rise and a delayed fall (vide chart, April 5th to 10th.

5. Tuberculin (T.R.) markedly improved the symptoms and an index raised by a *coli* infection, showing that the effect is not entirely a specific one. Although the patient was tuberculous there were no tubercle bacilli in the urine.

6. The present patient seems defective and abnormal as regards her immunization machinery, and the index in other cases may react differently.

7. The curve of the antitryptic index is perhaps more staid and moves in straighter lines than a chart of the opsonic index, probably because it is so much more accurately measurable. This consistent behaviour of the curve is, to my mind, strong evidence of the reliability of this technique.

8. In this chart the index displays a quality which simulates inertia, for whenever it falls suddenly it is apt to overshoot the normal line and to become a *negative index*, like a heavy, quickly-moving lever.

9. This throws some light on negative indices, which as isolated observations were less intelligible.

10. This method becomes a possible competitor with the opsonic index as another means of measuring immunization response.

REFERENCES.

[1] Golla, Proceedings of the British Medical Association, Belfast, July, 1909. [2] Hort, Diagnosis of Malignant Disease by Estimating the Antitryptic Power of Cancerous Serums, *Clinical Journal*, June, 1909; and Proceedings of the British Medical Association, Belfast, July, 1909.

ON THE VALUE OF TEST MEALS AS A GUIDE TO INFANT FEEDING.

BY RONALD CARTER, M.R.C.S., L.R.C.P., M.D.BRUX.,
KENSINGTON.

THREE years ago I started "infant consultations" in North Kensington (Notting Dale), with the help of the Kensington Health Society.

The mothers brought their infants once a week for advice because they were not progressing favourably. The majority were breast-fed, and, as is usual in these cases, friends told the mothers to wean the child. It seemed to me it would be a help in the elucidation of this problem to follow Professor Budin's plan and weigh the infant before and after a breast feed, and so discover at any rate the quantity of milk it obtained, and I have found this method to be of great practical importance. The number of breast-fed infants that "go wrong" is really amazing, and I feel quite certain that a large proportion of the deaths from gastro-enteritis attributed to bottle feeding are in reality breast-fed infants who have been artificially fed as a last resort. I have watched several of these cases, and I can positively say that the initial disturbance in nutrition started whilst the infant was breast-fed, and that artificial feeding was only resorted to when the degree of wasting had become noticeable to friends and relations.

In the case of breast-fed infants there should be some sort of co-ordination between the supply on the part of the mother and the demand on the part of the infant. A strong infant, by reason of its active powers of suction, affords the appropriate stimulus for a parallel activity on the part of the secreting gland, while the feeble nursling, on account of its indifferent powers of stimulation, excites little reaction in the breast. In a considerable proportion of cases there is no co-ordination between the supply and demand; sometimes there is too much milk, and sometimes not enough. Apart from the physiological test—namely, the progress of the infant—there is no way of finding out how much milk an infant receives unless we weigh the infant before and after feeding on very accurate scales; the amount consumed is estimated by noting the difference in the two weighings. This method is known as the "test feed." The following case illustrates its practical application:

A woman came to my consultations with a very wasted infant aged 2 months, and weighing 6 lb. She had fed it entirely on the breast, and assured me that it obtained the milk because it sucked for about ten minutes and then fell asleep. A "test feed" was arranged, and two hours after the last feed the infant was put to the breast. The scales proved that it obtained no milk at all. Milk could, however, be easily squeezed from the nipple, showing that an adequate supply was present. I ordered the mother to give 1 oz. of cow's milk with 1 oz. of barley water alternately with the breast feedings. During the following week the test feed showed that the infant obtained ½ oz. from the breast, and the child had increased 4 oz. in weight. She continued to feed in this manner for another week and the test feed then showed that 1 oz. was obtained from the breast, the child having gained another 5 oz. in weight. At the end of a month's treatment 2 oz. was obtained from the breast, and the child had gained nearly 1 lb. The cow's milk was now discontinued and the child was fed entirely on the breast till it was 8 months old.

Irregular feeding is a frequent cause of vomiting and diarrhoea in breast-fed infants; the scales have often shown what small quantities these infants obtain, and when the mothers are told to feed "by the clock," the result is that the vomiting and diarrhoea cease and the child obtains often double the quantity of nourishment from the breast. When irregular feeding is persisted in, the child begins to waste, the mother then commences bottle feeding, with of course a bad result; should such a case end fatally, the doctor in attendance, if he had not inquired into the previous history, would naturally assume that bottle-feeding was the cause of death.

Another interesting observation that the test feed has disclosed is that among breast-fed infants it is not always those who are inadequately fed according to our accepted scientific data who suffer from wasting, but often those who receive an adequate or even excessive amount. I have notes of at least forty cases in which the infant appeared to thrive and maintain a good weight curve on half the quantity of food that it should normally obtain; for