UNIVERSITY COLLEGE HOSPITAL MEDICAL SCHOOL
(UNIVERSITY OF LONDON).

TELEPHONE NO.
EUSTON 5361
EXTN. 20

UNIVERSITY STREET, W.C.1.

Department of Morbid Anatomy
PROFESSOR G. R. CAMERON,
F.R.S.

4th June, 1954.

My dear De,

I received your note of the 31st May this morning. Do not worry about feeling stagnant. So far as I can see there is not the slightest sign that that is the case. That last paper of yours in the Journal of Pathology on haemorrhage and cholera is an excellent piece of work, as I happen to know from the remarks made by Professor Oakley, and the other paper you had in the B.M.J. or the Lancet - I have forgotten which - is also a good piece of work. You are doing far more than most places in India ever achieve and I am very proud of the way in which you are running your department and inspiring your pupils. I hope that you will be promoted to take Professor Tribedi's place when he goes. You seem to me to be the obvious choice, but of course one never knows about these things and university and government politics are often disappointing. However, I feel that it would be very difficult to pass over your claims, especially since you have published such a lot of good work since you took over your Chair.

I wish you could come back and have a year with us, or at any rate part of a year, and enjoy a rest and contact with other people. We are doing very well. There is a lot of excellent work coming out and being prepared for publication. I am most pleased with Basu Mallik and Gupta from New Delhi is shaping very well. We have two new people promised for next October but there will be the inevitable departures.

Thank you for your kind remarks about the two papers. The one in the B.M.J. has been particularly successful judging by the requests for reprints from all over the world, and the people who have written to me about it. I am giving the first Matthew Stewart Lecture at Leeds on the 17th June and I expect that will be published sooner or later. It is called "The Exploration of the Cell" and it will be largely based on the work going on in my department. It will be an amusing experience in a way because Professor Matthew Stewart will actually be there together with his wife. In July I am taking part as one of the openers in a Ciba Foundation Symposium on ageing and I am also lecturing to the International Medical Students' Society on cirrhosis of the liver. All of these things take time to prepare but they are useful exercises in catching up with the literature and collecting one's own thoughts.

My mother is much the same. She has her bad weeks and then improves. You would see a great change in her. She is very slow in her speech, very forgetful and at times wanders a great deal, but she often asks about you and she was delighted when she received your last letter. She has very little strength to support herself and is confined to her room in the nursing home.

How is your family getting on? I expect your children are growing rapidly and you are seeing great changes in them year by year.

I shall not be able to send a paper to the Association of Indian Pathologists this year but I will try and do so in the following year, unless of course I find August a much quieter month than I anticipate. It is the only month in the year to which I look forward to doing things which particularly interest me apart from routine and supervision. If I get a week's quiet I may be able to send you a paper to read this year, but I fear that will not be the case.

With all good wishes,

Ever yours,

G Rbameron

Professor S. N. De,
45, Harnison Road,
Calcutta, 9.

FROM THE MASTER

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OXFORD
POSTCODE OX1 3TU
TELEPHONE 43182

31 May 1977

Dr S N De
P-100-A, C.I.T. New Road,
Scheme 52
Calcutta 700 014
India

Dear Dr De,

I am thinking of writing a history of bacterial toxins and propose to work myself into the subject by writing an account of why it was that although cholera toxin was the first to be postulated (Snow, 1849?; Koch, 1884) it was the last to be discovered (S N De, 1959). Diphtheria toxin was postulated by Loeffler in 1884 and demonstrated four years later by Roux and Yersin; tetanus toxin was postulated by Nicolaier in 1885 and demonstrated by Faber in 1890. I think that it took so long for cholera toxin to be demonstrated for a number of reasons:

(1) Koch had insisted both at the first and the second cholera conferences in Berlin, 1884 and 1885, that death from cholera was not due to the dehydration and thickening of the blood but due to a systemic action of the toxin. This, I think, led most investigators in the decades that followed always to test for toxicity by the parenteral route, which caused a great deal of confusion.

(2) In the early thirties, this confusion was stabilised by the acceptance of the erroneous idea that the cholera toxin was its endotoxin.

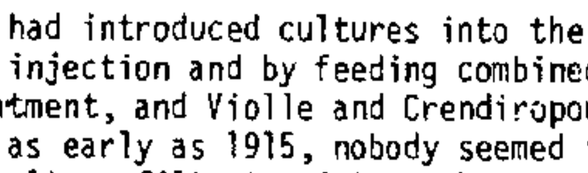
(3) In the early fifties a further confusion was added by the mucinase hypothesis.

(4) Although Koch himself had introduced cultures into the small intestine, both by direct injection and by feeding combined with alkali and opium treatment, and Violle and Crendiropoulo had invented the ligated loop as early as 1915, nobody seemed to think of putting cholera culture filtrates into such preparations. Nobody, that is to say, until you finally did so in 1959.

I think your work was of tremendous significance and most serious researchers on cholera throughout the world acknowledge this. But I am told that you were disappointed at your famous experiment because you thought that you had not really established your point. I would be most grateful if you would be so kind as to tell me whether this was so. I should also be very glad to have your comments on the other points I have made in this letter.

It is a great honour for me to be in correspondence with you. I have made many visits to the Cholera Research Laboratory in Dacca, and I hope to make some more. Next time I do so I would like to stop over in Calcutta and pay my respects to you.

Yours sincerely,



W E van Heyningen
Reader in Bacterial Chemistry
Sir William Dunn School of Pathology
University of Oxford

CENTRAL DIAGNOSTIC LAB.

S. N. DE, M.B.E. (CAL.) PH.D. & SC. (LONDON)
PROFESSOR-DIRECTOR OF PATHOLOGY CALCUTTA MEDICAL
COLLEGE & BACTERIOLOGIST TO GOVT. OF WEST BENGAL (RETD.)

P. 100-B, C. I. T. SCH. 51, PAIKPARUKUR, ENTALLY
CALCUTTA-700014
PHONE 44-1502

PATHOLOGICAL REPORT

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NAME .....

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TYPESCRIPT OF S. N. DE'S HANDWRITTEN LETTER TO W. E. VAN HEYNINGEN, OXFORD UNIVERSITY*

Dear Dr. van Heyningen,

It has been a pleasure to receive your letter of May 31 for which I am most thankful.

The reasons as noted by you for delay in discovery of cholera enterotoxin are to my mind, quite correct. The early French work on ligated loop was not extended further and was forgotten. When we used the ligated loop in 1953, we actually rediscovered the technique only to be surprised about 7 years later to find it accidentally as an abstract in Tropical Diseases Bulletin, 1915. I have acknowledged this in my book Cholera, its pathology and pathogenesis, *Oliver & Boyd 1961. If the French work on loop technique was more widely known and/or used, cholera enterotoxin would have been discovered much earlier - the only question then was to strike at the proper culture medium.*

On my side I was satisfied about the cholera enterotoxin. My disappointment came when I tried to extend the work to its natural conclusion - viz. to prepare a preventive cholera toxoid. This was due to

(1) My failure to concentrate the toxin

(2) replacement of typical strains of cholera vibrio by the so-called El Tor vibrios which are poor toxin producers and which, I think, are mutated forms of V. cholerae.

(3) My failure to preserve the toxicity of the toxigenic strains. Workers in developed countries cannot imagine how difficult it is to carry out and continue research work without willing personnel and without equipments in an undergraduate teaching (Path & Bact) cum hospital pathology, bacteriology & histology dept. in a country like ours.

My teacher the late Sir Roy Cameron was a source of constant inspiration to me and his encouragement kept up my spirits when low. His death put the last nail on my struggle against all these odds.

I retired from service in 1973 at 58 and I am now running a grocer's shop - viz. a clinical diagnostic laboratory at my residence. I have taken it as a hobby which keeps me fit and fairly busy. I have at least this consolation that I am giving some service to the public switching over to an applied branch of my broad subject of pathology.

Yours is a name much familiar to me as I have read at one time with great interest your chapter on toxins. Please do try to contact me when you pass through Calcutta and kindly let me know beforehand. It will be a pleasure and honour to meet you in person.

With kind regards,
Yours sincerely,

**This letter was written in response to van Heyningen's letter of 31 May, 1977 which is printed on p. 689 of this issue.*

NOBEL SYMPOSIUM TALK BY S. N. DE*
(AUGUST 6–11 1978)

Chairmen and friends,

Arne Tiselius, in his pontifical address on ''Priorities in Scientific Research'' commented ''when discoveries are published in scientific literature, they are presented in a form which does not tell us very much about how things really happened''.

Let me begin with the untold story of how we hit upon the rabbit ileal loop technique from which have stemmed all the present important and interesting developments on enterotoxins. It will appear as reminiscences of a retired person that I am and I hope you will allow me the indulgence.

In our first animal experiments, we opened the abdomen of . . . . rabbits under local anaesthesia and introduced heavy culture of *V. cholerae* mixed with mucin into the lumen of the small intestine. Little symptoms were noted but some of the animals were seen dead in 3 to 4 days. At autopsy the huge caecum of these rodents which normally contains pasty semisolid material was found distended with semiliquid faecal matter. We realised why previous workers had failed to produce cholera in rodents – the fluid that pours out in the small intestine finds a wide and comfortable accommodation in the caecal backwater for absorption and does not find its way out. We at once proceeded to produce localised cholera in a short ligated segment of the small intestine, by-passing the intervention of the caecaum, and the trick worked.

**This is the unedited text of De's talk at the 43rd Nobel Symposium on Cholera and Related Diarrhoeas held at Stockholm, August 6–11, 1978. The original text found with De's paper contains markings indicating several possible deletions. The figures and tables referred to were presumably on slides and are not available. In reviewing this symposium, R. A. Finkelstein and M. Boesman-Finkelstein (Nature 1978, 275, 173) concluded by saying: "Participants were reminded of how far we have come in a short time by the presence of one of the Indian investigators, S. N. De (Calcutta), who in the late 1950s first showed that the symptoms of cholera could be produced in laboratory models by cell-free products of the cholera vibrio".*

At that time we were unaware of Violle and Crendiropoulo's 38 years old short paper in the proceedings of the French Societie de Biologie. About 5 years later when searching the old literature for preparing my monograph on cholera, I came across an abstract of the French work in the Tropical Diseases Bulletin. I have acknowledged this fact in my monograph. Ours was actually a re-invention and not a revival of the earlier French work, which had rapidly sunk into obscurity.

We were then labouring under the age-old belief that *V. Cholerae* produces an endotoxin. But sterile filtrates of ultrasonic lysates of washed vibrios failed to swell the rabbit loop. This led us to try sterile supernates from cultures in different liquid media. At last, 5 p.c. bactopeptone gave us the desired success. We attributed the fundamental pathology of cholera viz. outpouring of fluid into the small intestine to an exotoxin and called it cholera enterotoxin.

Since the termination of the last World War, some strains of *Escherichia coli* carrying certain antigenic markers have been

regarded as responsible for nursery outbreaks of infantile diarrhoea. In the early 1950s when the position of these enteropathogenic serotypes was at its height, we had the occasion to question the validity of this concept. At that time, we were much worried about our failure to isolate *Vibrio cholerae* from the stools of many of the cases admitted and treated as cholera - which yielded pure cultures of *Escherichia coli*.. We had just devised the rabbit ileal loop technique for *V. cholerae*. We chose to introduce 20 strains of *E. coli* isolated from 20 such cases of non-choleraic diarrhoea into the ligated loop, in the form of overnight culture in Dunham's peptone water medium of pH 8.4. We also included a second group of 20 strains from apparently healthy persons suffering from diarrhoea off and on, a third group of 20 strains from absolutely healthy persons and a fourth group of three serotypes 0-26, B60, H-IJ; 0-55, B59, H7, 0-III, B58, H2 received from Colindale, which had been isolated from cases of infantile gastroenteritis. The results are shown in Table I. We raised OB antisera in rabbits against the three serotypes and against two of our loop positive local strains, and examined how the sixty three strains react with the five antisera. The results are shown in Table 2. We concluded from our observations that serotype may not be the last word on the enteropathogenicity of a strain of *Escherichia coli*. Cholera enterotoxin was yet to be born, it was not even conceived and the question of toxigenicity of the coliform strains was beyond imagination at that time.

Our observations were soon confirmed by Taylor and her colleagues and by McNaught and Roberts who found that serotypes from actual cases of diarrhoea usually gave a positive reaction while the same serotypes obtained from healthy persons or from water sources gave a negative reaction.

The position of the serotypes appears to have been further jeopardised by the works of Sack and his collaborators and of others. It has been amply demonstrated that many strains of *E. coli* isolated from adult and infantile cases of diarrhoea produce enterotoxin but with an occasional exception, they do not come under the category of recognised serotypes. The enterotoxigenic strains are limited to about 10 nonclassical 0-groups with 24 H-groups.

The positive role of such toxigenic strains has been confirmed throughout the world. In the Far East, strains of *E. coli* from 12 outbreaks of enteritis in Tokyo have been studied by Zen-Yoji and colleagues and have been related to three nonclassical serotypes producing either ST or ST and LT. Reports of such enteritis have come from the Far West in Mexico in indigenous population and in travellers. In the Far South, isolation of enterotoxigenic coliform organisms has been reported from cases of summer diarrhoea in Pretoria children. In the North, what *Vibrio cholerae* could not do even after the seventh pandemic, enterotoxic commensals have done it. They have touched the shores of the Baltic sea and traveller's diarrohoea due to such organisms has been reported from Sweden.

Yet, even in more recent times at some places, there has been a preponderance of nontoxigenic classical serotypes,

(Table 3) over nonclassical toxigenic strain.

The ability of enteropathogenic serotypes to cause diarrhoea particularly in children, has in earlier years been confirmed by results of feeding experiments on babies and adults and of rise of haemagglutination titre against the incriminated strain. It must be agreed in the light of modern observations that a classical serotype is not necessarily pathogenic, but regarding those nontoxigenic strains which are still being isolated in large numbers in some places, the fundamental question is - are they responsible for the disease and if so, by what mechanism they are causing it.

Let us first ask ourselves if the procedures leading to detection of enterotoxin have been beyond criticism. The most important point to remember in this connection, is that the stability of the ent$^+$ plasmid is most unpredictable. The necessity of testing the strains as quickly as possible after isolation cannot be overemphasized. While some workers have done it, others have collected the strains and tested them after a variable period of preservation under different conditions (Fig. 1).

The cultivation of the organisms for production of enterotoxin *in vitro* has also varied widely. Different media have been employed by different workers – different volumes in flasks or tubes with or without shaking have been used - with no regard to surface-volume ratio. The temperature and period of incubation have varied and starting pH has remained unspecified. This last factor may be important as we found in our early work that pathogenic *E. coli* swells the rabbit loop when grown in peptone water or broth medium of pH 8.4 and fails to do so at pH 7.4.

Individual freedom of choice is understandable, as there is no standardized method of enterotoxin production *in vitro*. It is important to work it out. Even the Syncase medium in which strain 569B of *Vibrio cholerae* gives the maximum yield of enterotoxin, failed to stimulate production of any active filtrate with strains NIH 35 and 41 in the hands of Finkelstein and colleagues. We do not depend upon enterotoxin production for detection of *V. cholerae* enteropathy but for the other enteropathies due to the commensals, this is very vital.

As long as no standardized method is available, it may be worthwhile to do rabbit loop test with overnight culture of the strains in peptone water medium of pH 8.4. The results will show within the limits of animal experimentation, whether the strain is enteropathogenic or not. If positive, enterotoxin may be looked for in the filtrate from the loop fluid. By this procedure, pathogenic strains which may fail to produce enterotoxin *in vitro*, may yield it *in vivo*. When we first encountered strains of eltor biotype of *V. cholerae*, they were found to produce no enterotoxin in 5 p.c. bactopeptone in which cholera enterotoxin was first discovered. The strains when introduced into rabbit loops gave positive reaction, and sterile filtrates from the positive loop contents swelled the rabbit loop.

We have solely depended upon random selection of a variable number of colonies for detection of enterotoxin. A

supplementary procedure may be to look for enterotoxin in the stool filtrate. Evidence of toxicity was detected in 60-70 p.c. of cases of cholera by testing only one specimen of stool by Huber & Phillips, by Craig and by ourselves. It may be worthwhile to test at least three 6 hourly specimens. This procedure may spot, at least some cases which may be missed by testing enterotoxin production *in vitro* from a few random colonies from the stool. It will also prove at the same time that the toxin is being actually liberated *in vivo* in the patient's intestine.

When a classical serotype is isolated from a case of diarrhoea, its significance may be assessed from the results of the investigations summarised in Table 4.

Chairman and friends, before I conclude, I wish to make a few personal remarks. I have been dead since the early 1960's, I have been exhumed by the Nobel Symposium Committee and these two days with you make me feel that I am coming to life again.

I discontinued my work on cholera enterotoxin as soon as I felt that with the limited resources and technology at my disposal, it would be impossible for me to pursue it further as I desired. I have been glad to find that other workers - many of whom are here to-day, have stepped into the field which we were fortunate to open and have invested their untiring efforts, unflinching devotion and unlimited resources in men, money and advanced technology. I am sure the continued efforts all the workers around the world will open a new chapter in the Annals of Medicine and will contribute towards the progress and good of humanity.

★ ★ ★ ★ ★ ★ ★

De and *E. coli* Diarrhoea

Besides the heat-labile cholera toxin-resembling toxin, *E. coli* also produces a heat-stable diarrhoeagenic toxin, and some strains of *E. coli* that produce neither toxin are still capable of producing diarrhoea.

The diarrhoeagenic capacity of *E. coli* was in fact observed in Calcutta several years before the JHCMRT* arrived there. S. N De had observed that strains of *E. coli* isolated from cases of gastroenteritis caused fluid accumulation in his newly rediscovered intestinal loops. Perhaps it was that that inspired Kenneth Goodner, who, according to his fellow Inner Circle member Theodore Woodward,

> must be given full credit for the perceptive observation of the significance of *Escherichia coli* as a causative agent of human diarrheas when the cholera vibrio was reborn K. G. said simply to a group of experimental physiologists and clinical microbiologists, "Don't forget to put *E. coli* in the intestinal loop". This was his kind of catalytic thinking. My faltering memory places the approximate data of this prophecy in about 1960, during the formative years of the Dacca program.

But De's paper had been published in well-known and respected journal, the *Journal of Pathology and Bacteriology*, in 1956, for all to read, whether they were in Dacca, Calcutta or Philadelphia.

Excerpted from *Cholera: The American Scientific Experience, 1947-1980* by W. E. van Heyningen and J. R. Seal.

* Johns Hopkins Center for Medical Research and Training (Calcutta).

LAST MONTH

1977 JANUARY 1977

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1977 FEBRUARY 1977

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NEXT MONTH

1977 MARCH 1977

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1136	+ + ±	589B — 2n	
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1154	+ + +-?		
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1156	+ + +-	359/64 2n	
1168	+ + ?	E3 2n	
1198	+ + +-	6E65 2.	
1191	+ + ∓	0E27 0g.	
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E1 0g	++ + ++	13 2n	
E3 0g	++ + ∓	11 2n	
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Physiology			

Notes