I would also point out that Gray *et al.* found fairly constant results from analysis of urea stibamine (Brahmachari), as shown in the accompanying table (the discrepancy in the antimony content of samples examined by them being due to varying amounts of the protective colloids present):

Carbon	Hydrogen	Nitrogen	Antimony
—	—	6·75	44·19
—	—	6·77	44·49
20·2	3·0	—	—
20·9	2·9	—	—
20·5	2·8	—	—
20·9	3·0	—	46·4
21·53	2·67	—	—
21·16	2·8	—	46·8
20·17	2·91	6·47	48·6

As already noted in NATURE[3], the divergent results obtained by different investigators were due to the fact that various manufacturers put on the market so-called urea stibamine which did not conform to my specification. This no doubt led to the conclusion of early workers that the so-called urea stibamine varied widely in its antimony content and was uncertain in its composition.

UPENDRANATH BRAHMACHARI.

19 Loudon Street,
Calcutta.

[1] NATURE, **144**, 1103 (1939).
[2] *Proc. Roy. Soc.*, B, **108**, 54 (1931).
[3] NATURE, **145**, 546 (1940).

Cultivation of Bluetongue Virus in Fertile Eggs produced on a Vitamin-deficient Diet

Two of us (R.A.A. and J.H.M.) have made many unsuccessful attempts to infect mice, guinea pigs, rabbits, hamsters, hedgehogs, and chicks with bluetongue virus. Mice and guinea pigs were not rendered susceptible by blockading the reticulo-endothelial system with India ink, by deep X-ray therapy, or by maintenance on a vitamin-deficient diet of autoclaved oats. Finally no multiplication occurred in normal developing chick embryos when virus was seeded on the chorio-allantoic membrane, or in the yolk-sac (method of Cox, 1938).

Various workers, particularly in the United States, have shown that deficiencies and intoxications can be produced in eggs, if the hens are put on appropriate rations. Thus, eggs may be obtained that are low in vitamins A, D, and E, pantothenic acid, riboflavin and manganese: if the diet contains much selenium, for example, the embryo is poisoned. It occurred to one of us (J.D.W.A.C.) that although bluetongue virus did not multiply in a so-called normal egg, yet it might grow in some of these 'pathological' eggs. We decided to infect eggs produced on a diet known to be deficient in the curled-toe-paralysis preventive factor, which is possibly riboflavin. Eggs laid by hens on a supposedly normal ration were also used, as a control.

In both groups, each hen had free access to water and oyster-shell grit and got about 1·5 oz. crushed yellow maize each evening, and a little more than 0·5 oz. chopped, fresh lucerne each morning. Dry mash was left before the birds all day. The two mashes were made up as follows:

	Deficient Mash lb.	Normal Mash lb.
Yellow maize meal	30	30
Pollard	30	30
Wheaten bran	20	20
Meat and bone meal (55% protein)	7·5	15
White fish meal (68% protein) ..	7·5	0
Lucerne meal (average quality) ..	0	5
Dried brewer's yeast	0	3
Common salt	0·5	0·5

Basing our calculations on American figures, we may assume that a hen given the deficient mash ate about 180 micrograms riboflavin per day. As a hen needs at least 230 micrograms daily for her eggs to develop properly, it is obvious that the deficient ration is low in riboflavin. Hens on the normal mash took in the adequate amount of approximately 270 micrograms riboflavin each day.

We used the above deficient diet because our colleague, J. J. Bronkhorst, had proved in chick experiments that the fish meal and the ration as a whole was very low in the curled-toe-paralysis preventive factor. It was he, too, who kindly supplied all the eggs for these experiments.

The virus for the 'pathological' eggs was a 10 per cent saline emulsion of infected sheep spleen, filtered through an 860 mμ gradocol membrane; 0·2 c.c. was inoculated into the yolk-sac of each egg containing a six-day-old embryo and the eggs incubated at 36° C. Serial passage was carried out every 4 or 5 days by subinoculating yolk-sac emulsion into the yolk-sacs of 'pathological' eggs.

With this technique the virus has been carried through 21 egg-to-egg passages with irrefutable evidence of multiplication. The yolk-sac, the embryo, and the chorio-allantoic membrane contain virus; in one experiment each was shown to hold between 100,000 and 1,000,000 infective doses for sheep.

After 4 passages in 'pathological' eggs, the virus was able to grow in normal eggs and could be maintained in them easily. At the present moment, the adapted virus is in its 37th normal egg passage. One experiment has shown that the virus content of normal eggs may be much lower than that of 'pathological' eggs.

As a rule, infected embryos die in 4 or 5 days, and are cherry-red when dead or dying.

Whether it really is the deficiency of riboflavin in the diet that has enabled us to propagate bluetongue virus in 'pathological' eggs remains to be seen, and experiments will soon be conducted to settle the question. At least twenty deficiencies and intoxications and combinations of these can probably be produced in eggs, and it would seem as if 'pathological' eggs might support the growth of other viruses that have not been cultivated in normal eggs. However, we may mention that neurotropic and viscerotropic strains of horse-sickness virus and the rickettsias of rat typhus and tick-bite fever multiply no better in 'pathological' than in normal eggs.

J. H. MASON.
J. D. W. A. COLES.
R. A. ALEXANDER.

Department of Agriculture and Forestry,
Division of Veterinary Services,
Onderstepoort,
South Africa.
April 24.

[1] Cox, H. R., *Pub. Hl. Rept.*, **53**, 2241–47 (1938).