

of these antigens, to the same titre. The titre observed was somewhat less than that obtained with the same serum and culture five years previously, a difference which might be due to the age of the serum. No tests of pathogenicity were performed. As so few of the Protozoa survive, it may be that cultures derived from these form a selected group—this possibility cannot be ruled out.

The extreme period of survival recorded is not in itself important; but it does suggest that cultures may be kept for as long as a year with every confidence of survival.

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¹ McEntegart, M. G., *J. Hyg.*, **52**, 545 (1954).

² McEntegart, M. G., *J. Clin. Path.*, **5**, 275 (1952).

A Toad (*Xenopus laevis*) without Hæmoglobin

DE GRAAF¹ has recently described a specimen of *Xenopus laevis* Daudin completely lacking hæmoglobin. Another specimen showing a similar condition was found in a practical class in this Department on September 20. The individual was a male measuring 6.5 cm. from the tip of the snout to the vent. Unfortunately it had been partly dissected under tap water and then left for some hours before my attention was directed to the specimen. It was one of a batch of toads delivered from the Trout Hatcheries near Kingwilliamstown in the Eastern Cape Province and had been in the laboratory for only five days before dissection.

As with de Graaf's specimen, the blood vessels were colourless or white except for the chromatophores in their walls; the heart was pale cream; the lungs and liver grey and, particularly striking, the kidneys cream. In contrast to the specimen found by de Graaf, the spleen appeared to be enlarged and was a light brown and not a slightly yellowish-white. The most remarkable feature, however, was that the gall bladder was rich in green bile pigment. This seems to suggest that extensive destruction of hæmoglobin had been occurring very recently.

de Graaf has shown that normal *Xenopus* are able to withstand functional elimination of at least 80 per cent of their hæmoglobin by carbon monoxide treatment. Although survival without hæmoglobin is clearly possible, he is careful to point out that this does not prove that an animal without hæmoglobin can be as active as a normal one. Nevertheless, in his discussion de Graaf appears to be assuming that his animal without hæmoglobin had been in that condition all its life, with the implication that growth to maturity in natural conditions is possible in the absence of hæmoglobin. The evidence that hæmoglobin destruction had only just been completed in the present specimen suggests a different interpretation—namely, that the totally anæmic condition is a pathological state which may arise after the adult has become mature. If this is correct then the existence of such individuals does not permit any conclusions to be drawn as to the importance of hæmoglobin for survival in natural conditions.

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¹ de Graaf, A. R., *J. Exp. Biol.*, **34**, 173 (1957).

Inhibition of Influenza Virus Multiplication with a Glucose Antimetabolite (2-deoxy-D-glucose)

OPTIMAL *in vitro* multiplication of influenza virus in the chorioallantoic membrane of the chick embryo is dependent upon the presence of glucose in the medium¹. Evidence on the importance of endogenous glucose in *in vivo* synthesis of influenza virus is lacking. The relatively low toxicity in both experimental animals² and humans³ of the potent glucose antimetabolite 2-deoxy-D-glucose suggested the feasibility of employing this compound in *in vivo* influenza virus infection. The present studies, undertaken in the intact chick embryo, demonstrate that the synthesis of influenza virus is markedly inhibited by 2-deoxy-D-glucose. Ancillary studies with *in vitro* systems show this inhibition to be reversible with glucose and therefore not related to permanent host cell damage.

The results of two representative experiments are shown in Fig. 1. In these experiments groups of six 10-day-old chick embryos were injected by the allantoic route with 12.5 or 25.0 mgm. of 2-deoxy-D-glucose or with water as a control. 18 hr. later, large inocula of influenza B (Lee) virus were introduced by the same route. These inocula were sufficiently large ($10^{7.5}$ EID₅₀, Exp. 559; $10^{8.0}$ EID₅₀, Exp. 560) to establish simultaneous infection of all susceptible cells to ensure a 'one-step' growth-curve. 8 and 24 hr. after the inoculation of virus, allantoic fluids from individual eggs were harvested and titrated with 0.5 per cent human 'O' erythrocytes for the presence of viral hæmagglutinin. The geometric means of titres within groups were calculated and are plotted in Fig. 1. At 8 hr., viral concentrations in control eggs were close to maximal, while yields in eggs injected with 2-deoxy-D-glucose were

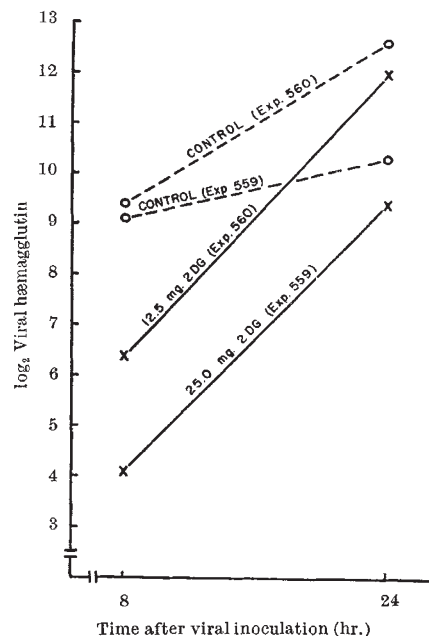


Fig. 1. Yields of influenza virus in allantoic fluids of chick embryos after prior injection of 2-deoxy-D-glucose (2DG). Geometric mean concentrations of virus derived from six individually titrated samples (allantoic fluids)/group. Chick embryos inoculated 18 hr. after injection of 2-deoxy-D-glucose with $10^{7.5}$ – $10^{8.0}$ per cent egg infectious doses (EID₅₀) of influenza B (Lee) virus