to the formation of benzylmercapturic acid. It should be mentioned, however, that glutathione failed to augment the synthesis of p-bromophenyl and l-α-naphthalenemercapturic acids in the rat, suggesting the inference that glutathione is not involved directly with the synthesis of these mercapturic acids *in vivo*.[5]

Summary. 1. S-benzylglutathione was synthesized and fed to adult rats. N-acetyl-S-benzyl-*l*-cysteine was isolated from the urine of these animals and identified by analysis. 2. The results suggest that the benzylated tripeptide was hydrolyzed in the rat to yield S-benzylcysteine which was then acetylated *via* the mechanism proposed by Knoop.[4]

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Development of Vaccinia and Variola Viruses in Embryonated Eggs at 28°C.

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At a temperature of 28°C the embryo in fertile 10-day hen's eggs ceases to develop but may remain alive for 48 to 72 hours, showing active movement on removal. By the 4th day, however, the embryo is usually inactive and autolytic changes may be apparent in the chorioallantoic membrane. It seemed of interest to determine whether certain viruses which are readily propagated in fertile eggs at temperatures favoring a normal development of the embryo could also be maintained at 28°C.

Accordingly, observations were made on the behavior of vaccinia and variola viruses in 10-day embryonated eggs held at a constant temperature of 28°C for 2 to 4 days in a humidified incubator. The chorioallantois was retracted from the shell membrane by suction and small unmeasured amounts of the respective virus suspensions in saline were introduced through a window in the shell. The eggs were generally opened on the 3rd day and the chorioallantoic membrane was removed for microscopic examination.

Vaccinia virus originally derived from the New York City Board of Health strain was established on the chorioallantoic membrane of embryonated eggs incubated at 28°C and maintained in 2 series of

[5] Stekol, J. A., *J. Biol. Chem.*, 1938, **122**, 333.

successive transfers through 20 and 10 passages, respectively. Elementary bodies were demonstrable by the silver impregnation method of Morosow in membrane films from 47 of 60 eggs used in the first passage series and in 21 of 28 eggs in the second series. The embryo was active on the 3rd day in 27 of the virus-positive eggs of the first series and in 13 of the second. Four bacterial contaminations were encountered in the 2 series of inoculations.

The number of elementary bodies in membrane films varied considerably from egg to egg; not infrequently, however, they were as numerous as in membranes from eggs incubated at 37°C. One titration of virus was made in embryonated eggs at 37°C, using a membrane removed on the 3rd day from the 16th passage at 28°C. The titer was 10^{-6}, the membrane inoculated with this dilution showing 5 discrete foci. A second titration was carried out in the skin of a rabbit, using a 10th passage membrane at 28°C; the limiting dilution was again 10^{-6}.

The development of vaccinia virus in the chorioallantoic membrane of embryonated eggs incubated at 37°C is accompanied by pathological changes, chiefly ectodermal proliferation and necrosis, which are clearly apparent macroscopically and microscopically. With the present strain of virus the embryo is usually dead by the 3rd day.

The most striking feature of the development of vaccinia virus in embryonated eggs incubated at 28°C was the lack of reaction in the chorioallantois. Many of the membranes which contained numerous elementary bodies appeared normal macroscopically. In some instances, particularly if retraction from the shell membrane was complete, minute foci were visible. These foci were generally so small that the membrane merely appeared clouded unless examined by low power magnification which brought out their discrete nature. Membranes examined on the 3rd day rarely showed any indication of necrosis. Those examined on the 4th day often showed autolytic changes whether virus was present or not. The histological findings were likewise atypical. The mesodermal blood vessels were regularly engorged and in some membranes, chiefly those which showed macroscopic foci, there were suggestive ectodermal thickenings. In the majority of the sections, however, the ectoderm was normal and intact. The mesoderm rarely showed an infiltration of phagocytic cells. The embryo in over half of the virus-positive eggs was normal in appearance and active on the 3rd day.

A strain of variola virus isolated in 1938 and carried through 43 egg passages at 37°C[1] was established in the chorioallantois at 28°C

[1] Nelson, J. B., *J. Exp. Med.*, 1939, **70**, 107.

and maintained for 8 transfers. Elementary bodies were demonstrable in the membranes from 17 of the 25 eggs employed but were usually less numerous than the elementary bodies of vaccinia. Fourteen of the virus-positive eggs contained an active embryo, in 2 of them after an incubation period of 4 days.

Most of the membranes inoculated with variola virus showed macroscopic evidence of a slight tissue reaction indistinguishable from that noted in some of the eggs inoculated with vaccinia and negligible in comparison with the reaction in membranes incubated at 37°C. At the latter temperature the development of variola virus is characterized by a focal epithelial hyperplasia which becomes confluent, resulting in a membrane many times thicker in cross-section than those incubated at 28°C. Histologically the membranes showed at most a moderate proliferation of the ectodermal cells with a few phagocytes in the mesoderm.

Summary. Vaccinia and variola viruses were established and maintained on transfer in the chorioallantoic membrane of embryonated eggs incubated at a temperature sufficiently low to prevent embryonic development (28°C). The number of elementary bodies present in the membrane on the 3rd day approximated that in eggs incubated at a temperature which favored growth of the embryo (37°C). At 28°C, however, the effect of the respective viruses on the membrane and the embryo as well as the cellular response of the former to them was significantly retarded.

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Identity of Natural and Synthetic Crystalline Vitamin B_6.

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Vitamin B_6 was first established as a component of the vitamin B complex by György.[1] It was subsequently isolated from natural sources by Keresztesy and Stevens,[2] Lepkovsky,[3] Kuhn and Wendt[4]

1 György, P., *Biochem. J.*, 1935, **29**, 760.

2 Keresztesy, J. C., and Stevens, J. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 64.

3 Lepkovsky, S., *Science*, 1938, **87**, 169.

4 Kuhn, R., and Wendt, G., *Ber.*, 1938, **71**, 780.