

was found to be about 150. Such a mixture is considered neutral—at least neither reagent is present in any large excess. It seems reasonable to suppose that within this zone, antibody and antigen enter respectively into the precipitate in the proportion of one molecule to one molecule, or one molecule to a small number of molecules. If it is assumed that the antibody is a globulin conjugate of the antigen, and contains a similar amount of arsenic (as would necessarily be true if there were no breaking up of the injected antigen), it can be shown from the amounts of arsenic and casein found in the casein-diazo-arsanilic acid solution, and the above ratio, that 10 cc. of antiserum should have contained about 20 mmg. (20×10^{-6} g.) of arsenic, an amount easily detectable. None was found. This would seem to cast doubt on the Buchnerian hypothesis.

In a recent paper Heidelberger and Kendall² similarly come to the conclusion that the injected antigen in one of their experiments must have split into at least two specifically reactive fragments, if the Buchnerian hypothesis be true.

However, from the amounts of arsenic and casein in the casein-diazo-arsanilic acid solution it is possible to arrive at a new estimate, namely, the number of diazo (haptens) groups in combination with one molecule of protein. For casein it was found to be 110, which in view of the roughness of the data may be considered a fair approximation to the theoretical value of 160. The questions now arise—with how many of these groups (haptens) must antibody react in order to produce precipitation, and how much is the injected antigen supposed to break up *in vivo* before entering into the specifically reactive hypothetical antibody-conjugate? The answers seem to be within reach of further experimental inquiry in which the authors are now engaged.

We are indebted to Mr. Saul Kamens for assistance in this work.

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Experiments on Gill Reduction in Neotonous *Triturus viridescens*.

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In certain regions neotonous individuals of *Triturus viridescens* are found in the same ponds with metamorphosed ones. Their gills

² Heidelberger, M., and Kendall, F. E., *Science*, 1930, **72**, 252.

persist after the body fin has been lost and the sex organs are well developed.¹ The object of these experiments was to find out whether such gills could be affected by environmental changes. If this occurred it would indicate that the hereditary background of these newts was different from that of those that lose their gills early.² Attempts to reduce the gills were made (1) by forced residence in a dry environment, (2) by implants of anterior pituitary lobe taken from gill-less adults from another region and (3) by implants of thyroid gland from the same source.

Gilled newts from Woods Hole, 6 to 10 cm. in length, and typical gill-less adults from South Hadley collected in October and November were used. Experiments were carried on through the winter and spring. Among 500 Woods Hole newts, 75% had gills on each side containing circulating blood. Their sex organs were partially or completely developed. The thyroids of 6 gilled newts suggested a state of rather low activity.³ A slightly less inactive condition obtained in thyroids of gill-less individuals from Woods Hole. On the other hand, the thyroids of South Hadley adults appeared active (compare adult *Ambystoma opacum*, Uhlenhuth³).

On October 14, 1930, 2 lots of Woods Hole newts with gills averaging 0.13 cm. in length were divided into 4 groups according to size. Twenty were kept in jars cushioned with moss occasionally sprinkled with water. Twenty others were kept in jars of water. Ten aquatic adults (South Hadley) were kept in moss-bedded jars and 10 in water. All were kept under the same conditions. Of the 20 gilled newts subjected to dry conditions, 10 were kept for 7 months or more. At that time the gills were shrunk but they expanded when moistened and circulating blood was visible within them.

In other experiments gilled newts kept in water were given bi-weekly grafts of anterior pituitary from donor adults (South Hadley) for 10 weeks. One set of controls was engrafted with intermediate and posterior lobes; another set was given no treatment. After the fifth anterior pituitary implant tail-fins appeared and the males developed characters typical of the breeding season. Within 20 days, 4 females laid eggs, continuing to deposit one to 8 at frequent intervals. Motile spermatozoa were found in the cloacae of the males. There was no noticeable change in the gills during the experiment nor for several weeks afterward.

¹ Noble, G. K., *Am. Mus. Nov.*, 1929, No. 348.

² Van Swinderen, M., *Tydschr. Neder. Dierk. Vereen.*, 1925, **19**, 141.

³ Uhlenhuth, E., *Roux' Arch. f. Entw. Mech.*, 1927, **109**, 609.

In the thyroid experiments, 2 thyroid glands from gill-less adults (South Hadley) were implanted bi-weekly into 20 gilled newts. No reduction of the gills was detected during the time that the animals survived, 10 to 27 days following the first transplant.

Throughout the experiments the gills appeared insensitive to pituitary and thyroid grafts although the condition of the sex organs, and the molting and emaciation indicated that the implants were potent in certain ways. However, during the winter and spring season here represented, the tempo of gill reduction was not accelerated by a dry environment nor by administration of endocrine glands.

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Homology of Prooestrous Bleeding in the Dog.

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The prooestrous uterine bleeding of dogs and menstruation of primates have been considered by some physiologists to be very similar and to represent in 2 classes of mammals the result of similar causative factors. (Heape,¹ Marshall.²)

We have obtained data from experiments conducted on dogs which we believe strongly indicate that the prooestrous bleeding of the dog is not homologous with the menstruation of primates. The evidence for this belief is as follows: (1) Ovariectomy of 5 dogs in the metoestrous phase did not result in external or profuse microscopical bleeding. In all 5 cases microscopical bleeding occurred as early as the 7th day and as late as the 16th day after ovariectomy. This bleeding was only of one or 2 days' duration. No external or microscopical bleeding was observed after ovariectomy of one immature dog and one dog in oestrus. Ovariectomy of monkeys results in external bleeding of about the same quantity as that seen at the normal menstrual period. (E. Allen³; Morrel *et al*.⁴; Saiki, in

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¹ Heape, W., *Quart. J. Micr. Science*, 1900, **44**, 1.

² Marshall, F. H. A., *Quart. J. Exp. Physiol.*, 1927, **17**, 205.

³ Allen, E., *Publ. Carnegie Inst. Washington*, 380. (Contrib. to Embryol., 19, 1.)

⁴ Morrell, J. A., Powers, H. H., Varley, J. R., and de Frates, J., *Endocrinol.*, 1930, **14**, 174.