

were first given 80 jack bean antiurease units into the ear vein. Then all 4 rabbits were given 50 units of soy urease intraperitoneally. The 2 unprotected rabbits died within 5 hours. The 2 protected rabbits were not affected.

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Sex of Parabiotic Twins in *Amblystoma Maculatum*.*

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After orthotopic transplantation of the gonadic primordia of *Amblystoma maculatum* (Shaw) the writer¹ found a differentiation of the gonad according to the sex of the donor rather than the sex of the host. When an ovary and a testis developed together in any host, however, the testis, whether that of the graft or that of the host, usually induced a modification of the ovary, reducing it in extreme cases to a rudimentary structure containing relatively few germ cells (freemartin ovary). These results were in marked contrast to those of Burns² who found only unisexual ($\delta\delta$ or ♀♀) combinations in 80 pairs of animals of the same species which had been joined in parabiosis in embryonic stages comparable to those used by the writer for transplantation of gonadic primordia. To determine whether the local (Buffalo) strain of *A. maculatum* would yield similar results under the conditions of parabiosis, 60 pairs were joined in the spring of 1931. Since the operations were performed late in the season on embryos of rather low viability, only 16 pairs survived to ages of 50 days or over. In 15 of these the gonads of both members were sufficiently differentiated to permit positive identification of sex. The combinations of sexes in these pairs were found to be as follows: 1 ♀♀ ; 6 $\text{♀♀}\delta$; 8 $\delta\delta$. In all 6 heterosexual pairs the ovaries of the female member had undergone modification under the influence of the male twin. The central ovarian cavity was absent and the cortex reduced, though in no case to a completely sterile condition. In 2 cases the development of hilar and medullary germ cells in one ovary of the female was

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¹ Humphrey, R. R., *J. Exp. Zool.*, 1929, **58**, 171.

² Burns, R. K., Jr., *J. Exp. Zool.*, 1925, **42**, 31.

sufficient to give that gonad the character of a retarded testis; the other ovary in each of these animals, however, exhibited only the usual freemartin state with little or no evidence of reversal. In the 8 pairs classed as ♂♂ the gonads of both members were in every case normal testes, showing no evidence of a reversal from ovaries. The apparent excess of ♂♂ pairs and the occurrence of but one ♀♀ pair are therefore regarded as a chance result, due to the small number of pairs surviving.

The occurrence of heterosexual pairs in the above experiment and their reported absence in the 80 pairs studied by Burns at first suggested to the writer a possible hereditary difference in sex-determining factors between the 2 strains of *A. maculatum* used (Buffalo strain, New Haven strain). This interpretation, however, was rendered untenable by the results obtained by Witschi, Gilbert and Andrew,³ who repeated Burns's experiments with embryos of the same strain (New Haven), and reported the occurrence of 18 heterosexual combinations among the 41 pairs examined. Since the modified ovaries of heterosexual pairs in the writer's material (and presumably those in the material of Witschi and his coworkers also) are usually very strikingly different from both the normal ovary and the normal testis, it seemed improbable that such gonads could have been overlooked or misinterpreted by Burns. The writer accordingly requested of Dr. Burns the opportunity to examine his material in order to determine if possible the reason for the apparent contradiction of his findings by the results obtained by other investigators. To this request Dr. Burns very kindly assented, and placed at the writer's disposal a large part of the material on which his report was based. Unfortunately a number of slides of this material had been lost through breakage, but since the pairs of animals thus unaccounted for in my examination lie in but a limited age-range in one series, their loss can not be regarded as affecting the validity of any conclusions from the remaining material, which included 57 of the original 80 pairs described by Burns. Among these are *all* of the 24 pairs kept to ages of 5 months or more.

Microscopic examination of this material forces one to the conclusion that reversal of sex has already occurred or is in progress (in 8 cases) in one member of all pairs primarily heterosexual. *The oldest and best differentiated pairs are invariably unisexual.* If the pair is ♀♀, the ovaries of neither member ordinarily show any

³ Witschi, E., Gilbert, W., and Andrew, G. O., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **29**, 278.

morphological evidence that reversal has occurred. A small number of the younger or less differentiated pairs classed by Burns as ♂♂ are, however, apparently of heterosexual derivation. In these, as a rule, the male member appears dominant, while the female member is either essentially indifferent, or, if more advanced in development, shows a varying degree of reversal of the ovaries to testes. The majority, in fact, possess a testicular structure in such large parts of their gonads that these can only be classified as testes. Their derivation from potential ovaries is indicated by the occurrence of sterile regions, or regions in which the germ cells are chiefly cortical in position. None of these gonads is a typical freemartin ovary, however, and in none is an ovarian cortex at all prominent. This marked suppression of ovarian features, together with the characteristic testicular structure of large portions of these gonads, naturally led to their classification by Burns as retarded testes.

In only one pair was there found any indication of the reversal of a male to a female. In this pair the gonads of one member are ovaries, either modified or in a very early stage of differentiation. Germ cells are few, but are chiefly cortical in position and some are in oöcyte stages. No ovarian cavity is present. The 2 gonads of the supposed male member of this pair are quite unlike in structure. One resembles an early testis, much retarded, with its few germ cells chiefly in a central location; the other is more like an early ovary, since its germ cells are chiefly in a cortical position, and a central cavity is lacking. Aside from this pair, which is scarcely past the indifferent stage, no pair gives evidence of reversal of a male member to a female, though the proportion of ♀♀ pairs suggests the probable occurrence of such a reversal.

The ratio of 36 ♀♀ to 44 ♂♂ originally reported by Burns was interpreted by him as a 1:1 ratio. Burns admitted the probability that some pairs classed by him as ♂♂ might actually be indifferent, but called attention to the fact that their reclassification as such would tend merely to cause a closer approximation to the 1:1 ratio. In the 57 pairs of Burns's animals examined, the writer has found 6 pairs classed by Burns as ♂♂ which might preferably be classed as undifferentiated. The sex distribution of the remaining pairs may be stated as 26 ♀♀ : 8 ♀♂ : 17 ♂♂. This is far from the ratio of 1 ♀♀ : 2 ♀♂ : 1 ♂♂ required on the assumption that no reversal of sex has occurred in any pair. But since 7 of the 8 pairs classed as heterosexual are either essentially ♂♂ in character or show the male member apparently dominant, and since older pairs are invariably unisexual, it is probable that eventually these animals could only have

been classed as 27 ♀♀ pairs and 24 ♂♂. This is a close approach to the 1:1 ratio expected if reversal of heterosexual pairs should occur without a marked prepotency favoring either sex.

In view of the histological findings and sex ratios stated above, it seems logical to conclude that reversal of one member of each heterosexual pair has either occurred or was in progress when the animals were killed. Naturally the question arises as to why this should have occurred in Burns's animals and not in those of the writer or of Witschi and his co-workers. The answer to this, I believe, must be sought in the conditions under which these animals were reared.

The most striking feature of Burns's parabiotic twins is their marked retardation in development and differentiation. This in some cases, perhaps, is due to the handicap imposed by a very complete or intimate union of the 2 members. Burns⁴ states that these animals were fed chiefly on *Daphnia* and small ostracods. Either from inability to capture sufficient numbers of these small organisms or due to distaste for them, the parabiotic animals remained undernourished, as evidenced by the entire absence of fat from the fat bodies in practically all specimens. Normal well-fed larvae of *A. maculatum* of the New Haven strain, according to Twitty and Schwind,⁵ metamorphose at about 70 days after operation (at Harrison's Stages 26-31) and at that time range from 48 to 55 mm. in length. Burns's animals usually failed to metamorphose until 5 or 6 months of age and measured only 25 to 30 mm. in total length at that time. As a rule sex is readily distinguished by microscopic examination in normal *A. maculatum* larvae of 50 days. In Burns's animals the indifferent period is prolonged 30 days or more beyond this time, and some animals at 5 months of age are still difficult to classify as to sex.

Malnutrition even in unoperated larvae of *Amblystoma maculatum* prevents the normal increase in germ cells in the gonads, and causes them to remain for a longer time in the indifferent state. Such retarded gonads, when subjected to the influence of the gonads of a parabiotic twin, probably undergo reversal more readily and completely than would the rapidly differentiating gonads of a well nourished animal. In other words, sex-differentiation is probably subject to various modifying influences, and the influence of a parabiotic partner of another sex, superimposed on retarded development due to malnutrition, may differ markedly from the effect of the

⁴ Personal communication.

⁵ Twitty, V. C., and Schwind, J. L., *J. Exp. Zool.*, 1931, **59**, 61.

parabiotic twin alone. The effects of dietary and other factors on sex-differentiation in unoperated *Amblystoma maculatum* require further investigation in view of Burns's results and of the abnormal sex ratios reported by the writer⁶ in laboratory-reared animals of this species.

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Influence of Posterior Pituitary Extracts on Mineral and Water Exchange in Children.*

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The influence of posterior pituitary extracts on mineral and water exchange was studied in 2 boys aged 9 and 5 years. The environmental and metabolic conditions, except for changes induced during experimental periods, were kept as constant as possible throughout the study. Each day was divided into 4 six-hour periods beginning at 6:00 A.M. At the beginning of each period, the subject voided, was weighed, and then given an accurately prepared meal of known weight and composition. The 4 meals of the day were identical in every respect and were prepared from simple foods of relatively constant mineral content. Water balances were calculated by the method suggested by Newburgh, Johnston and Falcon-Lesses.¹ On ashed urine sodium was determined by the uranyl acetate method of Barber and Kolthoff² and potassium by the chloroplastinate method of Shohl and Bennett.³ In view of the short periods utilized, mineral excretion in the stool was disregarded and average urinary excretion on control periods was used as a base line for determining fluctuations from normal during experimental periods. The posterior pituitary preparation pitressin was administered in all instances by the subcutaneous route.

The results of the 2 experiments presented in Table I clearly dem-

⁶ Humphrey, R. R., *Anat. Rec.*, 1931, **48**, 22.

* This investigation was aided by a grant from the Research Fund of the Rockefeller Foundation.

¹ Newburgh, L. H., Johnston, M. W., Falcon-Lesses, M., *J. Clin. Invest.*, 1930, **8**, 161.

² Barber, H. H., Kolthoff, I. M., *J. Am. Chem. Soc.*, 1928, **50**, 1625.

³ Shohl, A. T., Bennett, H. B., *J. Biol. Chem.*, 1928, **78**, 643.