ments. This dose of antibiotic in a 200-g male rat was 40 to 60 percent lethal within 2 days with the syndrome reported by Philips *et al.* (*10*).

If antibody synthesis occurs in a manner similar to induced enzyme synthesis, then a specific messenger RNA is required. In theories based on clonal selection or genetics, the antigen would actuate the transcription of information contained in the genome; in theories not based on genetics the antigen would provide the information for specificity. The effect of actinomycin reported here suggests that a synthesis of a specific messenger RNA may be required prior to the synthesis of antibody. These results do not distinguish between the two theories but they do suggest that a DNA-dependent RNA synthesis occurs during the induction phase and when it is not synthesized, no antibody is produced.

Actinomycin D delays the immune response but does not inhibit the rate of antibody synthesis or the maximum titer of circulating antibody. This finding suggests that the delay is not due to a metabolism of the drug resulting in a dissociation of the antibiotic and DNA. Such a dissociation would affect the rate of antibody production as more and more of the drug is removed from the DNA. Replication of the DNA, however, would allow the DNA-dependent polymerase to function if there were no free actinomycin available to bind to the DNA. This would be consistent with the findings in vitro that concentrations of actinomycin that completely inhibit RNA polymerase inhibit DNA polymerase less than 5 percent (*2*). The dose effect observed implies that more than one round of replication would be necessary with larger amounts of the drug before the DNA could be used for messenger RNA synthesis.

CARL J. WUST
CLARA L. GALL*
G. DAVID NOVELLI
Biology Division, Oak Ridge National Laboratory,† Oak Ridge, Tennessee

References and Notes

1. C. J. Wust and L. Astrachan, *Federation Proc.* **21**, 380 (1962).
2. J. Hurwitz and J. T. August, in *Progress in Nucleic Acid Research*, J. N. Davidson and W. E. Cohn, Eds. (Academic Press, New York, 1963), vol. 1, p. 59.
3. G. Giudice and G. D. Novelli, *Biochem. Biophys. Res. Commun.* **12**, 383 (1963).
4. J. Sterzl, *Nature* **189**, 1022 (1961).
5. H. C. Nathan, S. Bieber, G. B. Elion, G. H. Hitchings, *Proc. Soc. Exptl. Biol. Med.* **107**, 796 (1961).
6. S. R. Schwartz and J. Andre, in *Mechanism of Cell Tissue Damage Produced by Immune Reactions*, Second Intern. Symp. Immunopathology, P. Grabar and P. Miescher, Eds. (Czechoslovakia Acad. of Sciences, Prague, 1961).
7. Obtained fresh from the University of Tennessee Agricultural Farm and washed extensively in 0.1*M* *p*H 7.0 phosphate-buffered saline prior to use.
8. We thank Merck Sharp and Dohme, West Point, Pa., for the actinomycin D.
9. T. Kameyama and G. D. Novelli, *Arch. Biochem. Biophys.* **97**, 529 (1963).
10. F. S. Philips, H. S. Schwartz, S. S. Sternberg, C. T. C. Tan, *Ann. N.Y. Acad. Sci.* **89**, 348 (1960).

* Summer trainee of the Oak Ridge Institute of Nuclear Studies. Present address: Biology Department, Rosary Hill College, Buffalo, N.Y. † Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

5 December 1963

Gynogenesis in Salamanders Related to Ambystoma jeffersonianum

Abstract. *The oocytes of naturally occurring triploid females of the* Ambystoma jeffersonianum *complex each contain 84 lampbrush chromosomes. This constitutes hexaploidy* (n = *14). The chromosomes are joined into pairs by chiasmata and form 42 bivalents. It is suggested that meiosis in triploid females is preceded by an endomitosis and the resulting sister chromosomes synapse to form pseudo-bivalents. Sperm from diploid males stimulate development of the triploid eggs but do not contribute chromosomes to the triploid nucleus. Bivalents in the oocytes of triploids have twice as many chiasmata as the corresponding bivalents in diploid animals. Such chiasmata cannot result in genetic recombination.*

Natural and continuing populations of triploid females belonging to the *Ambystoma jeffersonianum* complex occur in northeastern North America, where they intermingle with certain populations of the diploid species *A. jeffersonianum* and *A. laterale* (*1*). The triploid females are characterized by having erythrocytes which are considerably larger than those of the diploid animals (*2*). They are accordingly described as large-celled females. The diploid chromosome number for both *A. jeffersonianum* and *A. laterale* is 28 (*1, 3*). Preparations of mitotic chromosomes from the larvae of large-celled females show 42 chromosomes per somatic cell (*1*): We have studied the lampbrush chromosomes in growing oocytes of large-celled females in an effort to determine the mechanism of meiosis in these animals. Chromosomes of the lampbrush type are a feature of amphibian oocytes. They are at a stage corresponding to early diplotene of first meiosis (*4*).

The animals used in this study were collected in Dodge County, Wisconsin; in Washtenaw County, Michigan; and in Sussex County, New Jersey. The blood cells of animals which were thought to be triploid were measured and compared with those of diploid females of *A. jeffersonianum* and *A. laterale*.

Erythrocytes were obtained by cutting the web of one hind foot. The cells were suspended in amphibian Ringer solution and mounted under a coverslip supported with petrolatum. The erythrocytes were drawn with the aid of a camera lucida at a magnification of about 900 times. The drawings represented optical sections through the two longest axes of the ellipsoidal cells. The area of a drawing was circumscribed twice with a planimeter and the total area was divided by 2 to obtain a mean value. At least ten cells from each animal were measured. Cell size proved to be an effective indicator of ploidy. The mean area of erythrocytes from the four triploid females collected in Washtenaw County was 1130 μ^2. The corresponding value for the two females of *A. laterale* collected in Dodge County was 730 μ^2.

The ploidy of each animal was further checked by measuring the amount of DNA per erythrocyte nucleus. For DNA measurements animals were killed and about 0.25 ml of blood was removed from the heart and mixed with 5 ml of 0.01*M* citric acid. The concentration of red blood cell nuclei in this suspension was determined from three separate hemocytometer counts and was adjusted to about 2×10^6 nuclei per milliliter. The diphenylamine reaction (*5*) was used to determine the total DNA in a 1-ml sample of the final suspension. Sperm DNA (*6*) at a concentration of 200 μg/ml in 0.01*N* NaOH was used as a standard. The amount of DNA per nucleus was calculated from

Table 1. The amount of DNA per erythrocyte nucleus and the number of bivalents per oocyte nucleus for two females of *Ambystoma laterale* and six triploid females.

Animal	County	DNA per nucleus ($\mu g \times 10^{-6}$)	Number of preparations	Number of bivalents*
		Triploid females		
AJ 1	Sussex	Not known	4	42 (2)
AJ 2	Washtenaw	156	15	42 (3)
AJ 3	Washtenaw	159	8	42, 35†
AJ 4	Washtenaw	176	10	42, 40‡, 39‡, 41
AJ 5	Washtenaw	164	25	41, 37, 40, 39
AJ 6	Washtenaw	152	16	42 (5)
		Diploid females		
AJ 7	Dodge	102	8	14 (8)
AJ 8	Dodge	105	5	14 (5)

* Numbers in parentheses indicate the number of counts. † Two quadrivalents. ‡ One quadrivalent.

the number of nuclei and the total DNA in a 1-ml sample. This method was simple and gave reproducible results.

After an animal had been shown beyond reasonable doubt to be a somatic triploid, lampbrush chromosomes from its oocytes were examined. Preparations were made from oocytes of between 1.2 and 1.5 mm in diameter. Nuclei from oocytes of this size in triploid females contain lampbrush chromosomes with well-developed lateral loops. The techniques described by Callan and Lloyd (*4*) were used for isolating and examining the chromosomes. Nuclei were removed from oocytes in a solution consisting of five parts of 0.1*M* KCl and one part of 0.1*M* NaCl and then transferred to observation chambers containing 0.02*M* 5 : 1 K and NaCl for removal of their membranes. Saline at a concentration of 0.02*M* was used as a medium since it produces dispersal of the stiff nuclear sap and spreading of the chromosomes over the bottom of the observation chamber. In 0.02*M* saline, however, much of the ribonucleoprotein of the lampbrush loops goes into solution and some structures which might be of value as landmarks for identifying certain chromosomes are lost. Otherwise the chromosomes are well preserved. Chromosomes were examined, counted, and drawn in an unfixed condition with the aid of an inverted phase-contrast microscope and camera-lucida attachment (*7*). The final magnification of the camera-lucida system was about 260 times.

In Table 1 the data obtained from six triploid females and two diploid females are summarized. All 78 lampbrush chromosome preparations made from triploid females showed more than 14 bivalents. Chromosome counts were possible in 20 preparations. Counts of 42 bivalents were obtained from 12 preparations in which the chromosomes were well dispersed and unbroken by isolation procedures. In no preparation did the number of bivalents exceed 42. Lower counts were obtained from some preparations because the chromosomes were tangled and stretched and accurate counting was impossible. Quadrivalents were identified in three preparations. No trivalents or univalents were seen.

Table 2. Lengths in millimeters of drawn chromosomes. Measurements are shown of the chromosomes of four oocytes taken from two triploid females.

Chromosome	AJ 1		AJ 6	
	(1)	(2)	(1)	(2)
I	95, 109, 123	116, 129, 156	176, 185, 281	192, 195, 230
II*	80, 85, 86	89, 90, 98	160, 161, 165	172, 177, 185
III	69, 76, 76	72, 74, 79	143, 149, 160	142, 151, 160
IV	64, 65, 66	69, 71, 71	117, 120, 123	128, 130, 131
V	56, 59, 60	64, 65, 66	98, 98, 116	119, 121, 127
VI	44, 45, 54	49, 49, 50	82, 87, 94	105, 113, 115
VII	35, 38, 42	47, 47, 49	65, 74, 80	95, 98, 98
VIII	35, 35, 35	39, 42, 44	60, 60, 61	89, 95, 95
IX	30, 30, 31	38, 39, 39	58, 59, 59	86, 86, 89
X	26, 26, 30	35, 36, 38	54, 55, 58	73, 79, 83
XI	21, 21, 24	27, 32, 34	50, 51, 54	66, 70, 71
XII	17, 19, 20	25, 27, 27	33, 42, 50	57, 61, 61
XIII*	11, 12, 13	20, 22, 23	28, 28, 32	49, 50, 54
XIV	9, 9, 11	18, 19, 20	20, 22, 27	37, 44, 46

* Matched by landmark structures.

Camera-lucida drawings were made of the chromosomes of four undamaged, well-dispersed preparations, each of which showed 42 bivalents. For each bivalent drawn the lengths of both homologs were measured in millimeters with an opisometer, and they were then averaged. The bivalents from each preparation were then grouped in threes in order of decreasing length (Table 2). That such a grouping is representative of hexaploidy in the oocytes of triploid females is confirmed by the incidence of landmark structures. Chromosome XIII can be identified by two loci which carry structures similar in appearance to the spheres described by Callan and Lloyd (*4*) on the lampbrush chromosomes of *Triturus cristatus*. Chromosome II can be identified by a pair of large lateral loops that are located about half way along its length. In all preparations, three bivalents of chromosome XIII were identified. In the preparations in which the chromosomes were well dispersed, three bivalents of chromosome II were identified.

We conclude from our observations that at diplotene of first meiosis the oocytes of triploid females contain 42 bivalents and are therefore hexaploid.

The number of points of contact between chromosomes forming bivalents was estimated in four preparations from four triploid females and in five preparations from two diploid females of *A. laterale*. We have assumed that these points of contact represent chiasmata, but we cannot be sure that all of them are true chiasmata which persist through to the first meiotic metaphase (*8*). The mean number of chiasmata per oocyte in triploid females was 414; that in oocytes of diploid animals was 68. In triploid animals each bivalent showed approximately twice as many chiasmata as a corresponding bivalent from a diploid animal.

The male salamanders of the *A. jeffersonianum* complex are necessary for the reproducing of the triploid females. Either kind of male, *A. jeffersonianum* or *A. laterale*, can fulfill the male role. The young of triploid females resemble the female parent rather than the male. In an experimental mating of an *A. jeffersonianum* male with a triploid female from a population of *A. laterale*, the young look like young triploid females from the *A. laterale* population. They do not look like young *A. jeffersonianum* of the same age, nor like young triploid females from an *A. jeffersonianum* population. A more de-

tailed account of these observations will be published elsewhere (*2*).

With regard to the mechanism of meiosis in triploid females, we suggest that early in oogenesis an endomitotic division takes place and produces oocytes with 84 chromosomes. Homologous chromosomes do not generally synapse. Instead, the sister chromosomes produced in the endomitosis become associated with one another by chiasmata and form 42 "pseudo-bivalents." After the two meiotic divisions, ova with 42 chromosomes are produced. Sperm from males of *A. jeffersonianum* or *A. laterale* stimulate these to develop although they do not contribute chromosomes to the triploid nucleus. The eggs develop into large-celled females with 42 chromosomes per somatic cell.

The occasional quadrivalent in the oocytes of triploid females could result from sufficient separation of sister chromosomes produced in the endomitosis to allow occasional synapsis homolog with homolog instead of sister with sister. However, the fact that no trivalents have been seen suggests that sister chromosomes resulting from the endomitosis do not separate widely.

The high incidence of chiasmata in triploid females cannot result in new genetic combinations since the chromosomes between which chiasmata form are presumably identical.

Similar meiotic and reproductive mechanisms have been described in the Lumbricidae by Muldal (*9*) and in the Enchytraeidae by Christensen and O'Connor (*10*). In those polyploid members of the Lumbricidae which reproduce parthenogenetically, Muldal (*9, 11*) has observed a premeiotic endoduplication which leads to the formation of pseudo-bivalents at first meiosis in the eggs. The chromosomes which form these pseudo-bivalents are joined to one another by chiasmata, but no multivalents have been seen. Muldal (*11*) suggests that the chromosomes are unpaired at the time of the endoduplication and that subsequent "pairing" takes place between sister chromosomes.

Christensen and O'Connor (*10*) described natural mixed populations of *Lumbricillus lineatus* consisting of diploid and triploid individuals. The triploid forms reproduce parthenogenetically, do not produce sperm, and lack seminal vesicles. Their spermathecae however, always contain spermatozoa. Sperm from diploid *L. lineatus* are necessary for the activation and normal cleavage of the eggs of triploids but do not contribute chromosomes to the triploid nucleus. Christensen and O'Connor described the relationship between triploid *L. lineatus* and diploid *L. lineatus* as "obligatory co-existence." A similar relationship would seem to prevail between the triploid females and diploid males of the *A. jeffersonianum* complex.

H. C. MACGREGOR
Department of Natural History, The University, St. Andrews, Fife, Scotland

T. M. UZZELL, JR.
University of Chicago, Chicago, Illinois

References and Notes

1. T. M. Uzzell, Jr., *Science* **139**, 113 (1963).
2. ———, *Copeia*, in press.
3. D. G. Humphrey, *Science* **128**, 304 (1958).
4. H. G. Callan and L. Lloyd, *Phil. Trans. Royal Soc. London, Ser. B* **243**, 135 (1960).
5. Z. Dische, in *The Nucleic Acids*, E. Chargaff and J. N. Davidson, Eds. (Academic Press, New York, 1955), vol. 1, p. 285.
6. Nutritional Biochemicals Corp., Cleveland, Ohio.
7. Cooke, Troughton, and Simms, Ltd., York, England.
8. I. D. Watson and H. G. Callan, *Quart. J. Microscop. Sci.* **104**, 281 (1963).
9. S. Muldal, *Heredity* **6**, 55 (1952).
10. B. Christensen and F. G. O'Connor, *Nature* **181**, 1085 (1958).
11. S. Muldal, personal communication.

14 November 1963

Gamma Hydroxybutyrate and Gamma Butyrolactone: Concentration in Rat Tissues during Anesthesia

Abstract. *Gamma-hydroxybutyric acid, when administered to animals or human beings, causes sleep. It is convertible to gamma-butyrolactone, which also produces sleep. Tissue concentrations in rats after administration of these two compounds show that the induced sleep is related to the concentration of the lactone in the brain.*

Gamma-hydroxybutyrate, a normal metabolite in brain (*1*), is apparently unique among natural intermediates in that it has anesthetic properties (*2*). Knowledge of the distribution of this compound in blood or tissues during anesthesia might be of use in understanding its mechanism of action. A primary question is whether the form of the compound directly related to sleep is the lactone or the anion. The ease with which lactone formation occurs, together with the markedly greater anesthetic properties of γ-butyrolactone (*3, 4*), suggests that the lactone might be the active form of the compound. This experiment was designed to expose any differences in the distribution and disposal of the two forms of the same compound in the tissues.

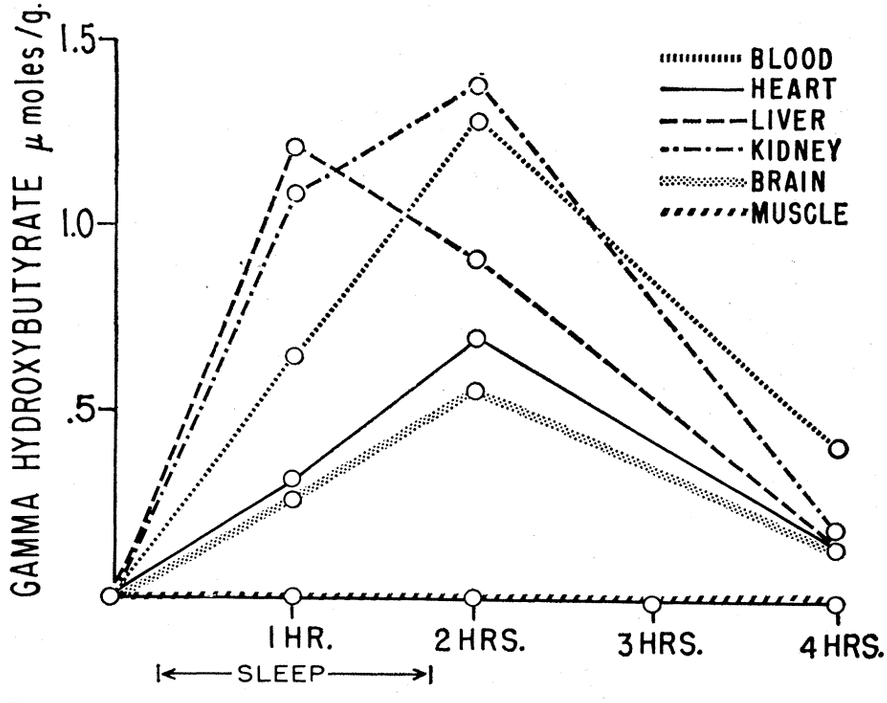

Fig. 1. Gamma-hydroxybutyrate concentration after intraperitoneal administration of sodium γ-hydroxybutyrate.