1947, v40, 417.

12. Deane, H. W., Shaw, J. H., Greep, R. O., *ibid.*, 1948, v43, 133.

13. Knobil, E., Morse, A., Greep, R. O., *Anat. Rec.*, 1955, v121, 324.

14. Weaver, J. A., *J. Endocrinol.*, 1956, v13, 189.

15. Jones, I. C., *Endocrinology*, 1949, v45, 514.

16. Lostroh, A. J., Woodward, P., *ibid.*, in press

17. Lever, J. D., *ibid.*, 1956, v58, 163.

18. Singer, B., Stack-Dunne, M. P., *J. Endocrinol.*, 1955, v12, 130.

19. Giroud, C. J. P., Saffran, M., Schally, A. V., Stachenko, J., Venning, E. H., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 855.

20. Rosenfeld, G., Rosemberg, E., Ungar, F., Dorfman, R. I., *Endocrinology*, 1956, v58, 255.

21. Selye, H., in Ralli, E. P., ed., Adrenal Cortex: *Transactions 4th Conf., Josiah Macy, Jr., Fn.*, Foundation Press, N. Y., 1953, p52 ff.

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Electrophoretic Studies on Serum Proteins of Young Germfree, Conventional and Antibiotic Treated Conventional Chickens.*† (23893)

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One of the characteristics of the germfree animal is a low level of antibody-containing globulin fractions. Thorbecke *et al.* found that in the germfree chicken a low level of gamma globulin persisted throughout the first 3 months of life. In the conventional‡ bird, during the same time period, this antibody containing fraction steadily increased(1). Meanwhile, we showed that conventional chickens treated orally from birth with 50 mg procaine penicillin G/kg diet develop certain organ characteristics which are qualitatively similar to those seen in germfree animals(2, 3). In essence, these consist of lower weight and less lymphoid tissue content of such organs which are normally in close association with an abundant microbial flora. Comparable changes have been reported by others(4, 5,6,7). Present evidence points to an influence of the antibiotic on composition and or metabolism of the intestinal flora as the most likely explanation of this effect on the host. It was therefore considered of interest to know whether oral administration of procaine penicillin G to young growing chickens, which was able to produce such definite "germfree-like" morphological features, would have an influence on the globulin fractions of the serum.

Methods. White Leghorn chickens of mixed sexes were used at 35, 75 and 125 days of age. The germfree birds were hatched into the Reyniers germfree system(8,9). After hatch the experimental animals were maintained in commercial brooders and in germfree units respectively. Percentage composition of diet (L-289F) was as follows: yellow corn 50, soybean meal 23, wheat midlings 9, casein 7, alfalfa 2, fish meal 2, meat scraps 2, minerals 4, vitamins and carriers 1. Throughout the experiment steam-sterilized diets were used (121°C, 25 min). Diet and tap water were offered *ad lib.* The present regimen allowed normal growth of both conventional and germfree animals. Procaine penicillin G was administered at 50 mg/kg diet. For further details, see Gordon *et al.*(3). All animal material indicated as germfree tested so up to moment of sacrifice, with the exception of the 125-day-old group. These animals became contaminated accidentally with a pure culture of *Micrococcus sp.*, 1 to 6 days before termination of experiment. Specific name of the contaminant was not determined. As previous experience has shown that in all probability no major changes in serum pattern can be ex-

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‡ Indicating animals of normal stock.

TABLE I. Serum Protein Fractions in Germfree (GF), Conventional (Conv.) and Penicillin-Treated Conventional (PP) Chickens (Sexes Mixed). Under heading Albumin, number of experimental animals is indicated. S.D.M. values are given of data expressed in mg %. The data were obtained with Antweiler's microelectrophoresis apparatus.

Age, days	Units	Albumin			α 1 globulin			α 2 + β globulin			γ globulin		
		GF	PP	Conv.	GF	PP	Conv.	GF	PP	Conv.	GF	PP	Conv.
35	%	78(8)	73(13)	73(9)	3	4	3	10	9	10	9	14	14
	mg %	2775	2509	2315	102	129	95	353	312	332	331	475	443
		± 94	± 55	± 89	± 8	± 15	± 24	± 31	± 26	± 21	± 21	± 39	± 23
75	%	77(5)	68(9)	68(11)	5	3	3	9	9	9	9	20	21
	mg %	2673	2712	2623	170	96	108	306	375	341	321	806	800
		± 79	± 84	± 115	± 31	± 14	± 19	± 27	± 16	± 25	± 43	± 85	± 55
125	%	74*(4)	63(11)	57(10)	4*	2	3	12*	12	12	10*	23	28
	mg %	2753*	2448	2450	160*	94	106	442*	461	506	397*	877	1171
		± 141	± 105	± 72	± 50	± 14	± 10	± 32	± 17	± 31	± 20	± 56	± 77

* Chickens became contaminated with a pure culture of *Micrococcus sp.* 1 to 6 days before sacrifice.

TABLE I (a). Selected Analysis of Differences in Table I.

	Albumin	P	α 1 globulin	P	α 2 + β globulin	P	γ globulin	P
Differences between groups	35 d GF *vs* Conv.	<.01	75 d GF *vs* Conv.	.23	120 d GF *vs* Conv.	.33	35 d GF *vs* Conv.	<.01
	GF *vs* PP	.03	GF *vs* PP	.01			GF *vs* PP	.01
	PP *vs* Conv.	.05	120 d GF *vs* Conv.	.50			75 d GF *vs* Conv.	<.01
			GF *vs* PP	.13			GF *vs* PP	"
	120 d GF *vs* Conv.	.05					125 d GF *vs* Conv.	<.01
							GF *vs* PP	"
							PP *vs* Conv.	.03
Difference with age					GF 35 d *vs* 125 d	.15	GF 35 d *vs* 125 d	.15
					PP 35 d *vs* 125 d	<.01	PP 35 d *vs* 75 d	<.01
					Conv. 35 d *vs* 125 d	"	75 d *vs* 125 d	.40
							Conv. 35 d *vs* 75 d	<.01
							75 d *vs* 125 d	"

pected within the first week after contamination, we have included these data in our results. Serum analysis was performed with Antweiler Microelectrophoresis apparatus using 0.2 ml samples. Total serum protein was determined using the Sargent Kagan Proteinometer.

Results are given in Tables I and I(a) while typical pictures of germfree and conventional chicken serum are given in Fig. 1. Fractions correspond to those given by Sanders *et al.* (10), and Deutsch *et al.*(11). It was, however, possible in practically all instances to establish the separation between fractions alpha 1 and alpha 2, while a clear-cut separation between alpha 2 and beta globulins was not always feasible, especially in older age groups. To avoid arbitrary values, alpha 2 and beta fractions are evaluated together, while alpha 1 globulin is given as such.

Results obtained with Antweiler Microelectrophoresis apparatus differ slightly from those obtained with the classical Tiselius technic. Bierring and Nielsen report that in human serum albumin tends to be about 10% higher, while beta globulin values are lower (12). These observations have been confirmed by us.

Gamma globulin values in germfree birds stayed low during the experiment, confirming earlier results(1). The conventional chickens already had a higher than germfree level at 35 days and showed normal increase with age. Penicillin-fed birds paralleled the untreated conventionals up to 75 days, but thereafter further increase in gamma globulin level seemed to slow down.

In many instances, a separation of gamma

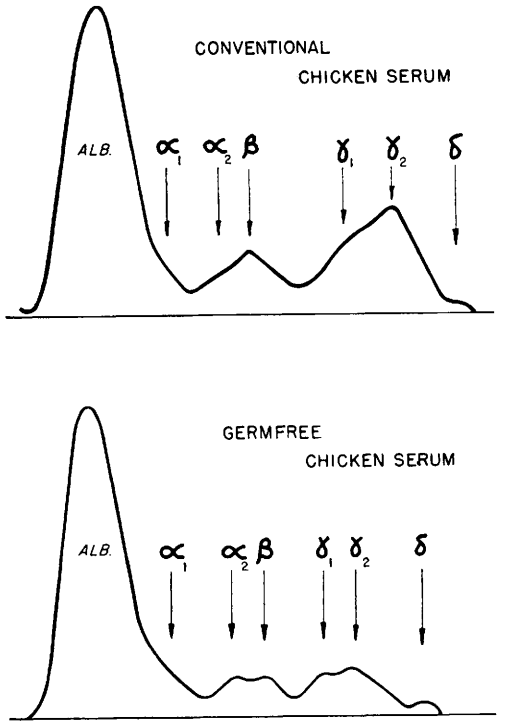

FIG. 1. Electrophoretic patterns of the serum of 75-day-old germfree and conventional chickens.

globulin fraction into gamma 1 and gamma 2 was possible. In all 3 experimental categories, the average amount of gamma 1 then comprised between ¼ and ⅓ of total gamma globulin fraction. No influence of age of animal on this partition was seen.

The alpha 2 + beta globulin fraction increased slightly with age and was apparently more or less alike in all 3 experimental groups. In the alpha 1 fraction, no obvious trends were seen. In the albumin fraction, however, germfree values tended to be higher than conventional and penicillin-treated, both in the 35-day and in the 125-day group.

For evaluation of these results, data on males and females were combined. This was done mainly because in the older age groups not enough germfree material was available to evaluate sexes separately. Analysis of data in the untreated conventional and penicillin-treated groups did not show any obvious influence of sex. This seems to agree in general with the observations of Vanstone *et al.* on chickens of comparable age groups(13). A possible leveling of gamma globulin values in the male after 9 weeks of age, as mentioned by these authors, did not show in the present results.

Discussion. Present data indicate that most or all antibodies which are formed as a reaction to bacterial stimuli are located in the gamma globulin fraction, especially since the increase of antibacterial antibody(14) and of gamma globulin with age go roughly parallel. In the germfree chicken, where no known stimulus of viable bacteria is available, a certain basic level of gamma globulin is still present, though the typical increase with age is virtually absent. The question whether this represents mostly "inert" gamma globulin or the product of an antibody response against unknown antigenic stimuli cannot be answered satisfactorily.

Morphological differences between germfree and conventional animals, such as reduced weight of small intestine and ileocecal lymph nodes, are well established at 35 days of age(3). In that case the neutralizing effect of penicillin on flora stimulation evidently makes itself felt from the start. However, antibody titers in normal conventional chicken are known to build up slowly(14), a fact which apparently is reflected in the gradual build-up of gamma globulin content over the germfree level. Here, the effect of the antibiotic seems to be virtually absent for the first

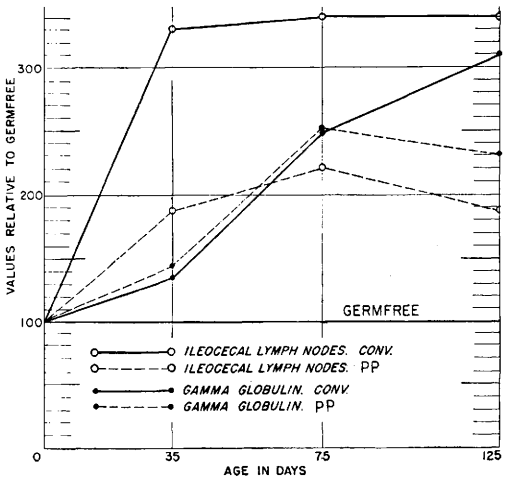

FIG. 2. Relative values of wt of ileocecal lymph nodes and of serum gamma globulin content of conventional (Conv.) and procaine-penicillin treated conventional (PP) chickens, setting germfree values at 100. All original data expressed in mg %.

3 months, while only at the age of 4 months, signs of a neutralizing effect became apparent (Fig. 2).

Evaluation of fractions gamma 1 and gamma 2 globulin was done wherever possible in the hope of finding one fraction containing mostly "germfree" gamma globulin along with another containing mostly flora induced gamma globulin. It was found, however, that gamma 1 and gamma 2 participated to about the same extent in all 3 experimental groups in all age classes investigated. There is a tendency for the germfree animal to have a somewhat higher serum albumin level. Our experiments have shown that this is definitely so in the rat and the same phenomenon manifests itself here in 35-day and 125-day groups. A possible explanation may be to regard the higher albumin content in germfree as compensating for the lower gamma globulin level to maintain the osmotic equilibrium.

As these experiments were mainly designed for a detailed study of morphological and related changes brought about by feeding of penicillin, the emphasis was placed on younger age groups and no data beyond 125 days are available. It is felt, however, that the influence of feeding penicillin on the gamma globulin level of the conventional chicken should be confirmed in animals of older age groups. Work along these lines is in progress.

Summary. The effect of feeding procaine penicillin G (50 mg/kg diet) on the electrophoretic pattern of serum of young conventional chickens was studied and compared with data obtained from germfree animals. While the effect of the antibiotic on morphological characteristics of conventional chicken made itself felt after one month, up to the age of 2½ months no differences were seen in the electrophoretic pattern. Both groups showed an equal increase in gamma globulin over the level found in serum of the germfree animal. At 4 months, however, the antibiotic-treated birds showed lower gamma globulin values than the untreated group, though the level was still substantially higher than found in germfree serum.

1. Thorbecke, G. J., Gordon, H. A., Wostmann, B. S., Wagner, M., Reyniers, J. A., *J. Infect. Dis.,* 1957, v101, 237.

2. Gordon, H. A., *Studies on Growth Effect of Antibiotics in Germfree Animals.* Lobund Inst., Univ. of Notre Dame, Ind., 1952.

3. Gordon, H. A., Wagner, M., Wostmann, B. S., *Antibiot. Ann.,* 1958, v58, 248.

4. Pepper, W. F., Slinger, S. J., Motzok, I., *Poultry Sci.,* 1953, v32, 656.

5. Coates, M. E., Davies, M. K., Kow, S. K., *Brit. J. Nutrition,* 1955, v9, 110.

6. Jukes, H. G., Hill, D. C., Branion, H. D., *Poultry Sci.,* 1956, v35, 716.

7. Hill, C. H., Keeling, A. D., Kelly, J. W., *J. Nutrition,* 1957, v62, 255.

8. Reyniers, J. A., Trexler, P. C., Ervin, R. F., Wagner, M., Luckey, T. D., Gordon, H. A., *Lobund Rep. No. 2,* 1949. Univ. of Notre Dame, Ind.

9. Reyners, J. A., *Proceedings of the 3rd Intern. Congress of Biochem.,* Brussels, 1955 (C. Liebecq, editor), Academic Press, New York, 1956, 458.

10. Sanders, E., Forest Huddleson, I., Schaible, P. J., *J. Biol. Chem.,* 1944, v155, 469.

11. Deutsch, H. F., Nichol, J. C., Cohn, M., *J. Immunol.,* 1949, v63, 195.

12. Bierring, E., Nielson, E., *Scand. J. Clin. and Lab. Invest.,* 1953, v4, 281.

13. Vanstone, W. E., Maw, W. A., Common, R. H., *Can. J. Biochem. Physiol.,* 1955, v33, 891.

14. Reyniers, J. A., Wagner, M., Luckey, T. D., Gordon, H. H., Lobund Reports No. 3, Univ. of Notre Dame, Ind., in press.

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